EXTENT OF DAMAGE TO STORED MILLED RICE

BY INSECT INFESTATION

by

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INTRODUCTION

Rice is one of the leading cereal grains, cultivated in almost every part of the world. In many developing countries this grain is the main daily food of the people. In those countries, people depend upon rice to fulfill about 50% of their carbohydrate requirement (Adair 1972).

Storage, one step in the normal postharvest pathway, is often the site of problems. However, proper storage can result in desirable changes in rice properties, such as improvement in flavor, odor or cooking quality (Barber 1972).

Rice is stored under various conditions for various periods of time before reaching consumers. Governments of some developing countries store large amounts of rice as national stocks to maintaining price stability at a reasonable level during the peak harvest and off-season and for political and security reasons. Unfortunately, poor storage conditions often allow deteriorative agents, such as insects, rodents, birds etc., to cause tremendous amounts of loss and damage to grain annually (Hall 1970).

Rough rice (with hulls still intact), is less susceptible to stored product insects than milled rice (Breese 1960, 1964). However, most of the developing countries store milled rice which is readily attacked by insects.

Percentage loss in storage is rather difficult to determine accurately. Various loss figures have been given and methods for assessing losses

have been published (Howe 1965b; Hall 1970; Adams 1970). However, completely satisfactory methods and estimates have not been developed.

Although methods for assessing losses of large grains, such as maize, peas or peanuts have been evaluated recently, according to Adams and Harman (1977), methods for maize are not completely suitable for wheat or other small grains. There is a need to assess losses in stored rice. Few studies have been concerned with losses in stored milled rice.

This study was to assess the losses and damage caused by two species of insects, either alone or in combination, in stored milled rice and to evaluate various loss assessment methods.

REVIEW OF LITERATURE

Definition of terms: losses and damage

Several workers have studied and reviewed losses in storage; however, criteria for defining losses are still nebulous.

Losses according to Schroeder and Calderwood (1972) are a direct result of the activities of several macro- and micro-organisms and occur because warehousemen fail to follow good storage practices. Abiotic factors, such as temperature and moisture may accelerate deterioration processes in storage since they interrelate with biotic and chemical variables (Sinha 1973).

Hall (1970) classified losses as weight loss, loss in quantity, nutritional loss and loss of seed. Recently, the National Academy of Sciences (1978) differentiated loss and damage. They defined loss as a reduction in weight of the food available for consumption, whereas damage was more closely related to physical spoilage. However, in a practical sense differentiation in such a manner is difficult, because both loss and damage interrelate.

Losses can be catagorized as quantitative or qualitative. Quantitative loss is loss of weight caused by living organisms as well as losses in moisture during the storage period (Pingale 1970; Adams 1976). This type of loss is easier to detect than qualitative loss. Many workers agree that the average post-harvest storage quantity losses is between 5 and 10% (Esmay 1970; Hall 1970).

Qualitative loss, such as loss of nutritional value of the grain, loss of energy or reduction of commercial value of the grain, is more difficult to assess. This type of loss is often not recognized and is

influenced by subjective judgments, local quality standards and the criteria of measurement used (Schroeder and Calderwood 1972; Adams and Harman 1977; NAS 1978).

Post-harvest losses which occur cannot be recovered, whereas preharvest losses or damage may be substituted by additional growth of the crop (Hall 1970; Adams and Harman 1977).

Damage caused by insect infestation

Insects are one of the most important factors causing grain deterioration. They consume all or parts of grain kernels and contaminate the grain with their excreta, webbing and castskins. Metabolic activity of insects may lower the grain quality by reducing the nutritional quality or viability of the seed (Hall 1970; Cotton and Wilbur 1974). Furthermore, they may produce favorable conditions for mold growth, bringing about heat damage, increasing moisture, off-flavor and total damage of the grain (Christensen and Kaufmann 1969; Christensen 1974).

About 30 species of stored-product insects have been found capable of infesting stored rice and rice products; however, only a few of these are a serious menace to rice in good condition (USDA 1964). The most destructive insects of stored rice are: the lesser grain borer, *Rhyzopertha dominica* (F.); the Angoumois grain moth, *Sitotroga cerealella* (Oliv.); the rice weevil, *Sitophilus oryzae* (L.); and the maize weevil *Sitophilus zeamais* Mots. (Schroeder and Calderwood 1972; Cotton and Wilbur 1974). Since these insects develop inside the grain kernels, their damage is not readily observed (Cotton and Wilbur 1974).

Intact hulls on rough rice kernels give good protection against insects (Breese 1960, 1964). Only Angoumois grain moth larvae and lesser grain borer are able to penetrate the hulls (Breese 1964; Prevett 1971; Schroeder and Calderwood 1972). Based on several samples taken from various local storages, Shahjahan (1974) reported 8.1% reduction of weight of rough rice due to Angoumois grain moth infestation.

The rice weevil infests rough rice only when there are defects in hulls, or untight hulls after blooming. Prevett (1971) also pointed out that even when a hull defect permits oviposition the adult progeny rice weevil may be trapped within the hull because the defect was not large enough to allow escape.

Whenever the hulls are defective or damaged by those insects capable of damaging the grain secondary insects,(such as the cadelle, *Tenebroides mauritanicus* (L.); the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); or several species of moths)are capable of feeding on the rice (Schroeder and Calderwood 1972). As a result of the various insect activities, serious loss and damage commonly occur.

Cogburn (1977) found that rice weevils were less damaging to rough rice (variety Vista) than Angoumois grain moths or lesser grain borers. After three generations, weight loss due to rice weevils was only 4% compared to 8 and 15% for Angoumois grain moths and lesser grain borers, respectively.

The susceptibility of milled rice to insect infestation is positively correlated with the proportion of broken kernels, degree of milling and moisture content of the grain (Prevett 1971; McGaughey 1974). Using

several species of insects reared on four varieties of rice with different percentages of broken kernels and different degrees of milling, McGaughey (1974) found that all insects produced more progeny in "reasonably well-milled" than in "well-milled" rice. Furthermore, rice varieties Nato and Vista were generally more susceptible to insect infestation than Belle Patna or Dawn.

Schroeder and Calderwood (1972) found that milled rice stored for a year lost more than 10% of the total weight due to insect attack. Each rice weevil during its development is able to eat 14 mg of milled rice kernel or about 70% of the total kernel weight (Mitsui 1970).

The maize weevil is generally known to infest maize in storage and in the field. This species may attack wheat, rice, sorghum or other grains. According to Floyd and Newsom (1959), *S. zeamais* (referred to as "*S. oryza*") in feeding preference tests, chose unpolished rice, sorghum, maize and wheat, in that order. They developed more rapidly in sorghum and unpolished rice than in maize. The greatest number of insects emerged from grain sorghum, followed by wheat, polished rice, rough rice and maize.

Kiritani (1965) reported maize weevils infesting milled rice in Japan, especially in areas where maize was not grown. Maize weevils were reported by McFarlane (1978, Personal Communication) in stored milled rice in Indonesia. He found maize weevil the predominant species and a serious problem in milled rice.

The red flour beetle, *Tribolium castaneum* (Herbst), is generally considered a secondary pest of stored grains (Cotton and Wilbur 1974), and does not primarily attack rice. However, under certain circumstances

it may become a pest either in rice or other grains. Prevett (1971) cited this species as a pest of rice bran and indicated it was an important deteriorative agent if rice was not well-milled or had a high percentage of broken kernels. Increasing percentage of dockage in wheat (dockage includes broken kernels, wheat chaff and dust) favors production of red flour beetle progeny (McGregor 1964).

Interaction among species of insects

In storage, interaction among species of insects is common and plays a key role in determining the size of their populations. Such association does not always benefit all species involved; often one species exploits activities of another and/or otherwise inhibits them. Competition and interference between the confused flour beetle, *Tribolium confusum* (J. du Val) and red flour beetle have been studied by several workers (Park *et al*. 1965; Inouye and Lerner 1965; Sokoloff *et al*. 1965). They agree that red flour beetles are more voracious than confused flour beetles, especially if reared in inadequate medium. In order to obtain essential nutrient requirements, red flour beetles cannibalized eggs and preadult stages of confused flour beetles (Sokoloff *et al*. 1965; Inouye and Lerner 1965).

Red flour beetles also prey on eggs and immature stages of other species of stored-product insects (Le Cato 1975a, 1975b, 1975c). Le Cato (1975c) studied the interaction of four species of stored-product insects reared on whole and cracked corn, at $28 \pm 1°C$ and $65 \pm 10\%$ r.h. over a period of 70 days. He found red flour beetles reduced populations of sawtoothed grain beetles and flat grain beetle, *Cryptolestes pusillus* (Schon.) in both whole and cracked corn.

Under certain conditions, populations of red flour beetles are suppressed by another species. Lefkovitch (1968) studied the interrelation among species of insects on small quantities of wheat and wheatfeed at 30°C and 70% r.h. over 6-wk period. Red flour beetles were inhibited by the presence of rusty grain beetles, Cryptolestes ferrugineus (Steph.) and also rice weevils. Populations of red flour beetles were reduced significantly when interacting with rusty grain beetles or rice weevils as compared to pure cultures.

In general, the association or interaction between species of insects in stored products has been considered to result in acceleration of the deterioration process and bring about greater damage to stored grain (Le Cato 1975b).

Interaction among insects and fungi

Evidence of the association among insects and certain species of fungi in stored grain is commonly found and has been reported by several workers. The activity of insects favors the development of an environment suitable for stimulating mold growth and development in grain. Agrawal et al. (1957) and Van Wijk et al. (1959) reported that granary weevils considerably increased the moisture content of the wheat in which they developed thus allowing storage fungi to grow. They also concluded that the granary weevil served as vector in carrying and spreading spores of fungi in the grain. Bronswijk and Sinha (1971) using wheat artificially infested with granary weevils and red flour beetles, found Penicillium spp. growing rapidly. The fungus did not grow in the media infested with rust grain beetles and sawtoothed grain beetles. They

assumed that granary weevils were responsible for an increase in moisture of the grain which, in turn, favored *Penicillium* growth.

Van Wijk *et al*. (1959) found that confused flour beetles are not affected by the presence of mold spores in the media. Increased populations of this species in wheat flour or wheat media suppressed the number of some species of fungi. It was presumed that quinones, secreted by confused flour beetles under certain conditions, were relatively toxic to fungi and inhibited their development.

The antagonistic effect of fungi on insects has also been reported. Development and reproduction of Indian-meal moth was hindered by the presence of certain species of molds, mainly *Aspergillus glaucus* and *A. flavus* groups (Abdel-Rahman *et al*. 1969). According to Bronswijk and Sinha (1971), granary weevils and lesser grain borers failed to reproduce on storage fungi as a sole diet.

The presence of fungi may favor development of certain insects. David *et al*. (1974) demonstrated that larvae of foreign grain beetles, *Ahasverus advena* (Waltl.), preferred *Cladosporium* sp., *Penicillium citrinum* and *A. amstelodami* and developed more successfully than in *A. niger*, *A. ochraceous* or *A. candidus*.

Invasion of fungi on stored grain may bring about undesirable effects, such as discoloration, mustiness, off-flavor, heat damage or production of mycotoxin (Christensen and Kaufmann 1974). One of the most important mycotoxins found in grain is aflatoxin, produced by *A. flavus* under certain conditions. Aflatoxin may be produced when the moisture content of grains, such as rice, maize or wheat is about

18.5% and temperature about 27°C (Christensen and Kaufmann 1974). Conditions favorable for production of aflatoxin by _A. flavus_ can be the result of insect development in grains.

MATERIALS AND METHODS

Simulated bags

Eight 70-l steel drums (approximately 40 cm dia and 70 cm long) were used to simulate bag storage. Each drum was provided with 20 holes, about 6 cm dia, positioned horizontally, with 4 rows of 5 equally spaced holes along axes at 90° to one another as shown in Figure 1. Holes were covered with 80-mesh brass wire screen to prevent insects from escaping and to allow air exchange between the atmosphere and drum (Fig. 1, B).

A 3 cm dia hole was drilled on top of the horizontal drum (Fig. 1, A), for introducing insects, and copper constantan thermocouple wires into the grain. Thermocouples were used to monitor the temperature of the grain at three different levels. This hole was closed with a rubber stopper and sealed with silicone caulking immediately after insects were introduced into the drum.

Three sampling ports were made on the lid of the drum by fitting 2.6 cm dia conduit connectors into 3 cm dia, vertically oriented holes (Fig. 1, C). These three openings were closed with rubber stoppers except during sampling. Sampling levels were designated as top, middle and bottom.

Each drum was filled with 54 kg of milled rice, and set horizontally on a 3-shelf wooden rack. Drums were held in a constant temperature humidity (CTH) room at 29 $\pm$ 1°C and 70 $\pm$ 5% r.h.

Rice

Nato, a medium grain rice, was obtained from a commercial rice miller in Stuttgart, Arkansas. Rice was reasonably well-milled (about

Fig. 1. Metal barrel (40 cm dia, 70 cm long) used for simulated bag storage of milled rice.(A) Port for insect introduction and thermocouple wires.(B) Ventilation holes (80-mesh brass screen). (C) Sampling ports.


A
B

80% bran removed) and had 25% broken kernels. Rice was milled to this specification to simulate the No. 2 Indonesian quality standard which is commonly found in the local market in that country. Rice was held in a freezer at -12.2°C for about 3 wk to eliminate any possible insect infestation and then transferred into cold storage (4°C) until used for the experiment. The rice, in jute sacks, was placed in heavy duty plastic bags while stored in the freezer and cold storage.

Prior to infestation, rice was tempered at room temperature for 48 hr. A double-cone mixer was used to mix rice thoroughly. Final moisture content after mixing was 13.5 ± 0.3%. Moisture was determined using a single-stage, air-oven method (130°C for 19 hr) described by Hart *et al*. (1959).

Rice, in the drums, was conditioned in the CTH room for about 4 wk prior to starting the experiment.

Insects

Maize weevils, *Sitophilus zeamais* Mots., and the red flour beetles, *Tribolium castaneum* (Herbst), were selected for this study because of their common occurrence in rice storage in Indonesia. Both species were obtained from the Stored Product Insects Laboratory, Department of Entomology, Kansas State University. Red flour beetles (RFB) were reared on whole wheat flour enriched with yeast at a ratio of 19:1. Maize weevils (MW), an Arkansas strain, previously reared on whole wheat kernels, were specially reared for 2 generations on milled rice with moisture content of 13.5%. Insects were reared at 27 ± 1°C and 67 ± 2% RH.

Unsexed MW and RFB, 2 - 4 wk old, were used to infest the rice. Two barrels each were infested with 300 MW (hereafter called S-system); two barrels each were infested with 300 RFB (T-system); two drums were infested with a combination of 150 MW and 150 RFB (S/T-system). The last two barrels were uninfested and served as controls.

Sampling and sample analysis

Sampling method. Samples were taken at the beginning of the experiment before insects were introduced. Subsequently, samples were taken at 4-wk intervals over a period of 24 wk. Samples were taken using a holed bag probe (about 2.4 cm dia and 75 cm long) of tube construction without partitions.

Three probes were taken at the top sampling port and two probes at each of the middle and bottom ports to obtain about 150 g samples from each level of the barrel. The probe was wiped with a cloth wetted with 80% ethanol to prevent mold cross-contamination of samples. Samples were placed directly in "zip-loc" plastic bags. Samples for moisture test were separated in the CTH room and the rest of the sample was returned to the plastic bags and saved for further analyses.

Moisture test. About 20 g rice from each sample was sieved using a No. 40 US standard sieve. Samples (5 to 10 g) of the sieved rice were placed in aluminium dishes for a single-stage air-oven moisture determination. Duplicate moisture contents (m.c.) for each level were determined and averaged.

The air-oven method used was as described by Hart *et al*. (1959) for wheat (130°C for 19 hr). Moisture content of dust was also determined

after 12, 16, 20, and 24 wk using the standard air-oven method (130°C for 1 hr). All m.c. were expressed as percent wet basis.

Number of insects. Numbers of live and dead insects were recorded at each sampling. The degree of infestation is reported as the number of insects per 50-g sample. In the S/T-system, besides total number of insects each species was counted and recorded separately.

Dust production. The No. 40 US standard sieve (425 μm openings), was used to separate dust from the kernels and insects. All fine material that passed through this sieve was considered as dust and included excreta and flour from endosperm. Dust was weighed to determine the amount recovered per 100 g of sample.

Loss assessment. Three methods were used to determine losses.

1. Flotation method. The flotation method used was developed as an adaptation of a traditional habit of peoples in rice eating countries (See Appendix B-1). Prior to cooking, the rice is commonly washed and rinsed to remove undesirable materials such as empty grains, dust or insects.

In this study, 50-g samples of rice with known moisture were washed and rinsed thoroughly with 500 ml of tap water. This wash/rinse process was repeated three times and then rice was drained and allowed to dry at room temperature for 24 hr. Samples were then weighed and the moisture determined.

The percentage loss was determined by comparing the initial weight to the weight after washing, on a dry matter basis. The difference in weight between the two divided by the initial weight, times 100% gave

the percentage dry matter weight loss. Losses determined by this method are hereafter called "F-loss."

Calculation of percentage F-loss is as follows:

$$\text{F-loss} = \frac{DW_i - DW_t}{DW_i} \times 100\%$$

where F-loss = % dry matter weight loss

DW_i = initial dry matter weight (before washing)

DW_t = final dry matter weight (after drying)

2. Volumetric method. A 50-ml graduated cylinder and small funnel were used. The method was calibrated with the standard apparatus for density test as described by Boerner (1916). Weight of 50 ml of rice was obtained.

A clean sample (free of insects and dust) was allowed to run through a funnel into the graduated cyclinder. The surface was smoothed in a standard manner. The weight of the rice was obtained using a torsion balance (accuracy 0.001 g). This process was repeated three times, and the mean weight used as the bulk density of the rice.

The dry matter weight for samples from each system and the control were used as an estimation of weight loss (hereafter called "V-loss").

3. Gravimetric method. After dust and insects were removed, 25 g of clean sample was put in an electronic seed counter (Syntron, Model EB 00 Style 2042) to determine the number of seeds. Based on this number the weight of 1000 kernels of rice was calculated.

The weight of 1000 kernels from each system was used to estimate weight loss (loss determined by this method hereafter called "G-loss").

Dry matter weight from each system was compared to that of the control. The formula is as follows:

$$\text{G-loss} = \frac{W_c - W_t}{W_t} \times 100\%$$

where G-loss = % dry matter weight loss

W_c = dry matter weight of 1000 kernels of rice in the control

W_t = dry matter weight of 1000 kernels of infested grain

This method is a simplification of the gravimetric method developed by Adams and Harman (1977).

Radiography test

The same samples used in density and 1000-kernel weight tests were used in the radiography test. X-ray has been widely used for detecting internal insect infestation in grains and for other purposes (Katz and Milner 1950; Mills and Wilbur 1967; Sharifi and Mills 1971).

The 25-g samples of clean grain were radiographed using a Hewlett Packard X-ray Unit Faxitron Series, Model 43804N, and Kodak Type M Industrex X-ray film. The radiographs were examined using an X-ray viewer and hand lens (2.5 X). Numbers of immature insects (larva and/or pupa) and damaged kernels found on radiographs were counted. Weevil-damaged kernels, with or without immature insects inside, were considered as damaged kernels. Results were expressed as numbers of immature insects and damaged kernels per 1000 kernels.

Odor test

Rice during storage usually acquires a specific flavor which is considered desirable by people in some rice-eating countries. However,

with the occurrence of insects or molds in the rice, off-flavor and/or odor changes frequently occur (Barber 1972).

Odor and other sensory tests (taste or touch when foods are eaten) are used for evaluating food quality (Larmond 1970). A multiple comparison difference analysis was used to test the quality of rice after it had been infested with insects and stored for 8 wk.

Samples from each system and control at each sampling level were placed in small bottles (about 50 g each), and covered with aluminum foil to avoid a bias during the test. Each bottle was numbered randomly and a standard rice sample (coded R) which had been stored at 4°C used as a reference.

Nine student panelists were selected from countries where rice is a part of the daily menu. Each panelist was given a questionnaire (Appendix B-2) and asked to compare each sample with the reference and score the difference.

The results of scoring were subsequently given numerical values 1 to 9 based on the following:

1- extremely better than R

2- much better

3- moderately better

4- slightly better

5- no difference

6- slightly inferior

7- moderately inferior

8- much inferior

9- extremely inferior

Analysis of variance and Duncan's multiple range test was used to analyze the data.

Mold invasion

Samples from each system were examined for fungal invasion. Fifty kernels were wetted with 75% ethanol, surface disinfected by shaking in 2% NaOCl for about one minute, and then rinsed with distilled water. Kernels were plated on MS4T culture medium (malt agar (Difco) with 4% NaCl and 200 ppm Tergitol NPX). Plates were incubated at room temperature until the fungal growth was mature enough for identification (approx. 5-7 days) using a binocular dissecting microscope.

In addition, sound and damaged kernels from S-system and the S/T-system were plated separately. For damaged kernels 5% NaOCl was used for surface disinfection. The mold invasion in each sample was expressed as percent invaded kernels.

Additional tests

At the conclusion of the experiment the following were measured: total weight loss, total dust recovered, total caked material, bulk density of cleaned rice and aflatoxin.

Dust recovery. Material remaining in each barrel (with the exception of caked material) was removed, weighed and sifted using a standard laboratory sifter with a No. 40 US Standard sieve (425 μm openings). All fine material that passed through the screen was weighed and used to find the percentage of total dust recovered from each system. Caked material was removed from barrels and weighed.

Bulk density. Clean rice from each system was measured using the standard procedure described by Boerner (1916).

Total weight loss. After the last samples were taken each barrel was weighed using an O'Haus scale (capacity 300 lbs and accuracy 0.25 lb). Weight of each barrel with rice at the beginning of experiment was also known. The difference between initial dry matter weight and end dry matter weight (after dust separation and removal of caked material) was used to estimate total loss after rice was stored for 24 wk.

Aflatoxin analysis. Because *Aspergillus flavus* was found in some of the rice samples, an analysis was carried out to check for aflatoxin contamination in the grain.

The thin layer chromatography method of Seitz and Mohr (1977) was used. Samples (50 g) of rice and caked material from each system were taken randomly, dried at room temperature and used for this analysis.

RESULTS AND DISCUSSION

Effect of storage time on moisture and temperature of uninfested rice

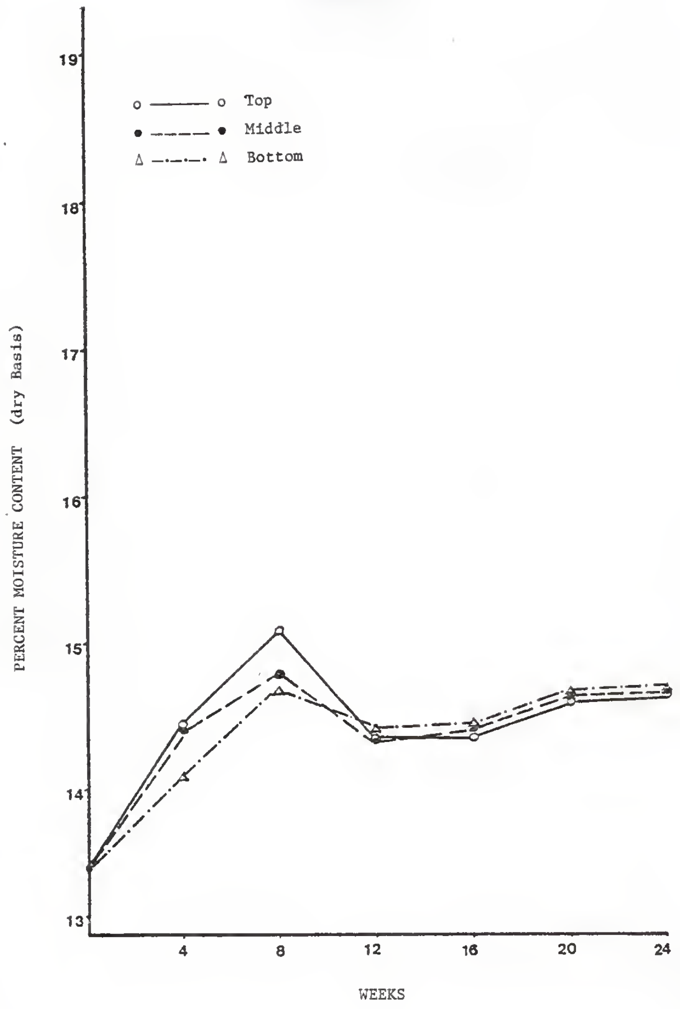
Initial m.c. of all rice at the beginning of the 24 wk storage was 13.5%. After 4 wk, average m.c. in uninfested rice had reached 14.3% and then gradually increased to an average of 14.7% at the end of 24 wk (Fig. 2).

There were no significant differences in moisture or temperature of uninfested rice between sampling levels after storage for 24 wk. Although there was a small unexplained fluctuation of m.c. of rice at 8 wk, thereafter moisture was unchanged and nearly uniform at all levels.

Rice had reached near equilibrium moisture content after 4 wk in the relative humidity of 70 ± 5% at 29 ± 1°C. Davey (1965) estimated the equilibrium moisture content of polished rice at 25°C and 70% r.h. at about 14.5%, approximately the same moisture as recorded in this study.

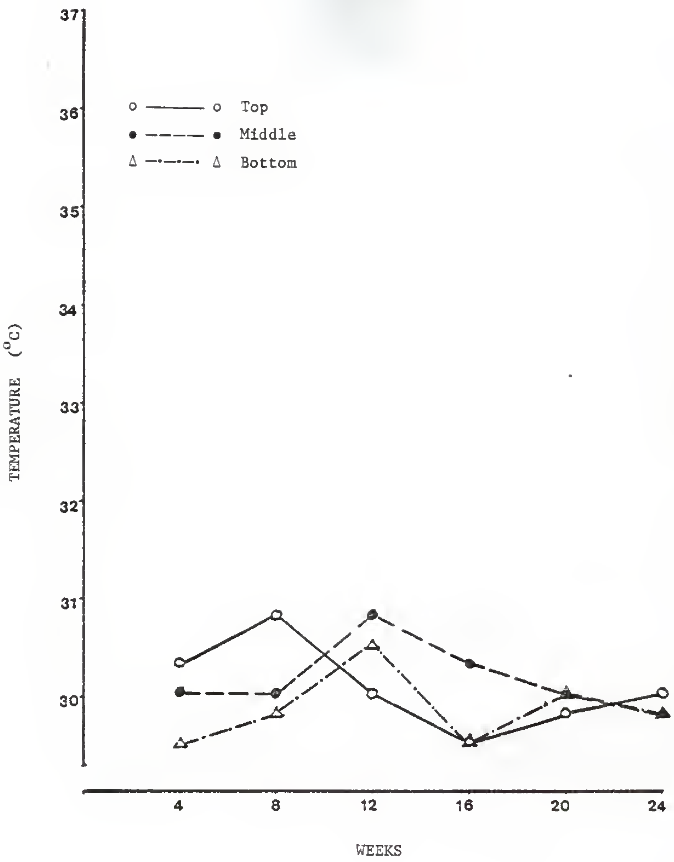
Rice temperature in the control was almost unchanged over 24 wk (Fig. 3) and had approximately the same temperature as the control room (± 30°C). Uniform temperature and m.c. at all three levels indicated that there was an effective air exchange between the atmosphere and the barrels.

Fig. 2. Percent moisture content of milled rice in the control (uninfested) stored at $29 \pm 1°C$ and $70 \pm 5\%$ r.h. during 24 wk.



Top
Middle
Bottom
19
18
17
16
15
14
13
PERCENT MOISTURE CONTENT (dry Basis)
4
8
12
16
20
24
WEEKS

Fig. 3. Temperature of milled rice in the control (uninfested) stored at 29 $\pm$ 1°C and 70 $\pm$ 5% r.h. during 24 wk.


37
36
35
34
33
32
31
30
Top
Middle
Bottom
TEMPERATURE (^{o}C)
4
8
12
16
20
24
WEEKS

Effect of *Tribolium castaneum* on stored rice

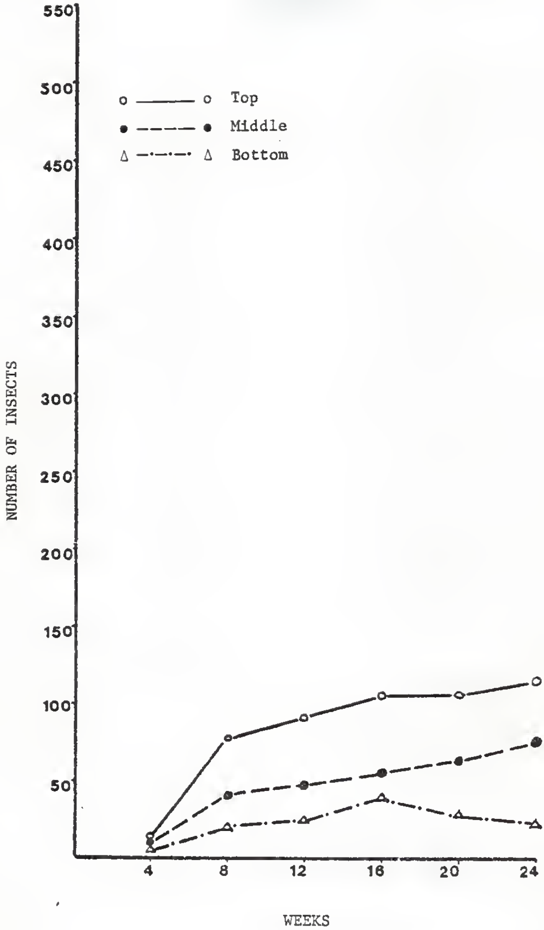
In general, little damage occurred in the T-system during 24 wk storage.

Number of insects (Fig. 4; Table A-1). A sharp increase in average number of insects was recorded at 8 wk, and thereafter gradually increased until the experiment was halted. Apparently, the RFB found difficulties in multiplying after 8 wk in milled rice; however, they still maintained their population. Only a few dead insects were recovered at each sampling intervals.

Presumably, because rice used in this study had a rather high percentage of broken kernels (25%) and was 80% bran removed, the RFB were able to survive and reproduce. According to McGaughey (1974) confused flour beetles preferred medium grain rice, variety Nato, with 74% bran removed over 85% bran removed; however, the number was low compared to primary feeding insects. Atmosudirdjo (1976) using rice with different percentages of brokens and degrees of milling, found more progeny in brown than in milled rice with 20% broken kernels.

The distribution of insects in the barrel remained constant from one observation to another. The insects recovered indicated that the upper layer was preferred. The findings of Surtees (1965) showed that the RFB tended to accumulate on the surface of stored grain, especially if grain temperature was above 15°C. They dispersed more if crowded (Surtees 1964c). Contrarywise, Howe (1951) and Sharangapani and Pingale (1957) pointed out that moving toward the bottom was the most natural movement of insects, including RFB, in wheat (bulk or bags).

Fig. 4. Number of insects per 50-g sample of milled rice infested with *Tribolium castaneum* and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.


550
500
450
400
350
300
250
200
150
100
50
Top
Middle
Bottom
NUMBER OF INSECTS
4
8
12
16
20
24
WEEKS

Dust production. (Fig. 5; Table A-2). The fine particles that passed through the No. 40 sieve were mostly grain dust and frass produced by insects. Little dust was recovered from samples before 12 wk. The amount of dust recovered from the infested samples was slightly higher than that of the control, indicating that RFB produced some dust. Correlation analysis confirmed that there was a relationship between insect numbers and dust produced ($r = 0.79$, $P = 0.05$), and the amount of dust increased as the storage time lengthened ($r = 0.83$). Bronswijk and Sinha (1971) found no correlation between dust and RFB populations in wheat artificially infested with granary weevils and RFB.

The amounts of dust recovered from each level were significantly different ($P = 0.01$), with most recovered from the bottom level at 24 wk. Presumably, insect activity and their movement caused the dust to settle to the bottom layer. Sampling procedure may have influenced the amount of dust in each sample, especially dust at the bottom level which was rather difficult to reach with the sample probe.

Grain moisture. (Fig. 6; Table A-3). The average m.c. of rice in the T-system increased gradually during the experiment, except at 8 wk when an unexplained sharp increase was noted.

Statistical analysis showed a high correlation between insect numbers and m.c. of the grain ($r = 0.72$), and between m.c. and grain temperature ($r = 0.82$). However, there was no significant difference in m.c. between the T-system and control. It has been noted by other workers that moisture may increase as a result of insect activity (Agrawal *et al.* 1958; Christensen and Hodson 1960).

Fig. 5. Amount of dust recovered from 100-g sample of milled rice infested with Tribolium castaneum and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

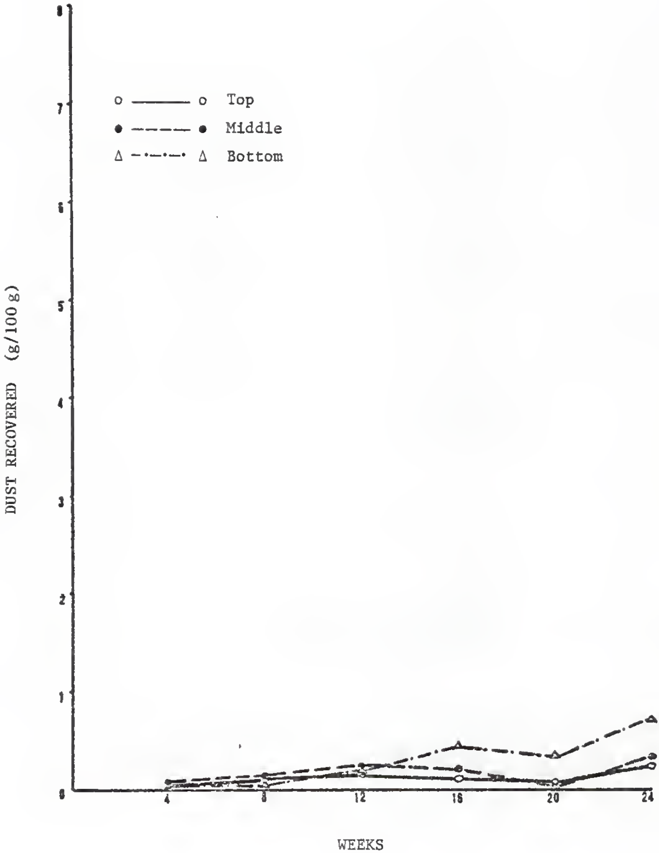
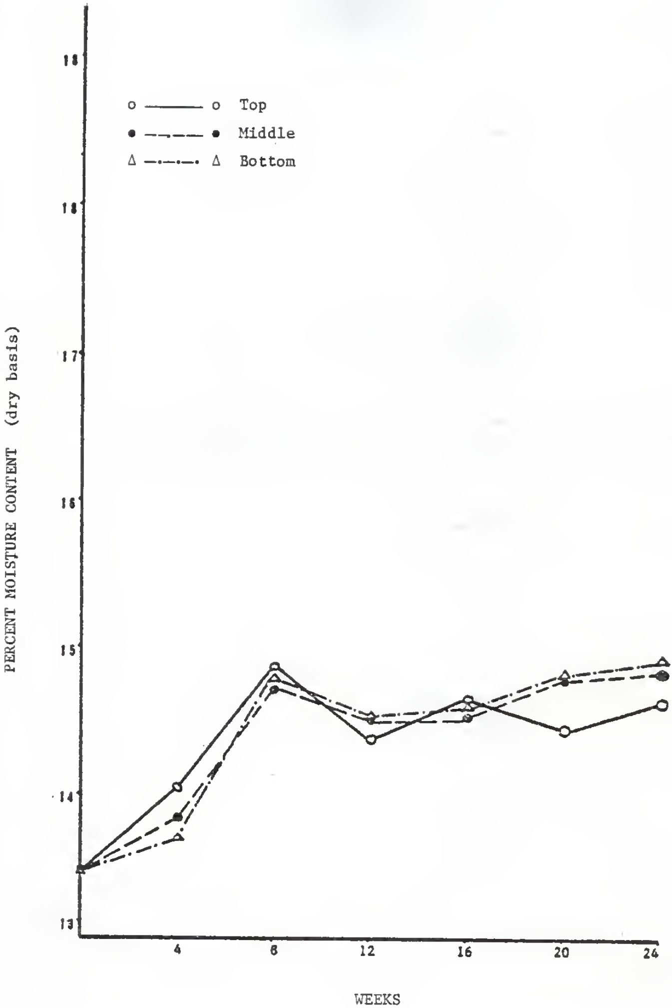

o —— o Top
● ---- ● Middle
Δ -·-·- Δ Bottom
DUST RECOVERED (g/100 g)
0
1
2
3
4
5
6
7
8
4
8
12
16
20
24
WEEKS

Fig. 6. Percent moisture content of milled rice infested with *Tribolium castaneum* and stored at 29 $\pm$ 1°C and 70 $\pm$ 5% r.h. during 24 wk.


o ——— o Top
● — - — ● Middle
Δ -·-·- Δ Bottom
PERCENT MOISTURE CONTENT (dry basis)
18
18
17
16
15
14
13
4
8
12
16
20
24
WEEKS

The bottom level had a higher m.c. than the other levels, and reached 14.9% after 24 wk. However, the average insect population at that point was lower than at the upper levels. Presumably, dust accumulation in the bottom was responsible for the higher moisture content of the rice. Moisture content of the dust separated from the rice was 23 - 24% after 20 wk of storage. In conditions where high-moisture dust surrounds rice kernels, the possibility for increased moisture in the rice in that area also exist. However, further study is needed to prove this assumption.

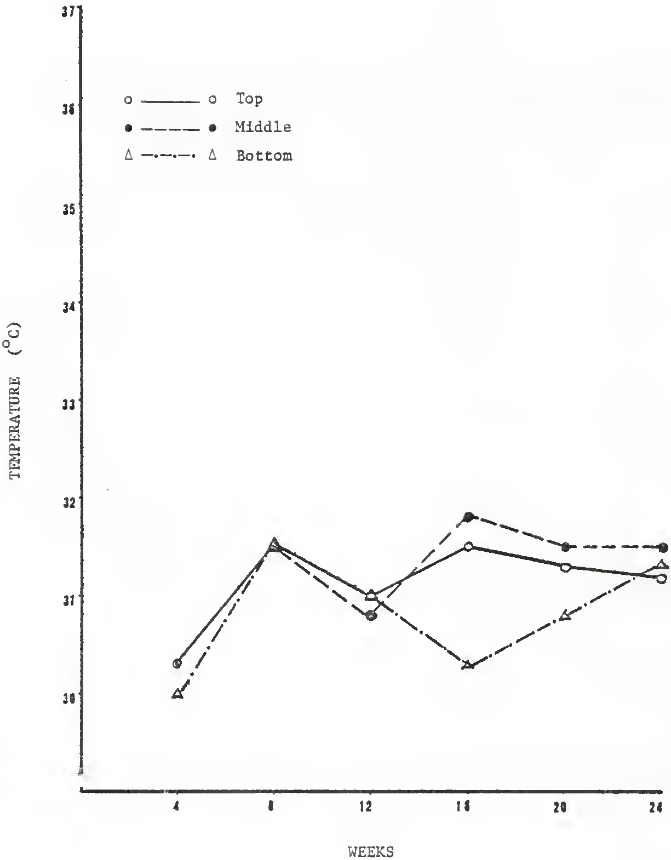
Temperature (Fig. 7; Table A-4). After 24 wk in storage, temperature in the T-system had increased between 1.2 - 1.7°C above room temperature and the control; however, the increase was not significant (Table A-4).

According to Cotton and Wilbur (1974), grain heavily infested by insects usually had a higher temperature. Sinha and Wallace (1966) found a high population of insects around hot spots in small, farm bins in Canada. Metabolic activity of insects had caused the increase in temperature of the grain.

Correlation coefficient between insect numbers and temperature in this study was significant ($r = 0.72$; $P = 0.01$). Since the increase in temperature was not significant, the population of RFB in this study apparently was not large enough to liberate heat faster than it was dissipated to the atmosphere.

Mold invasion (Table A-10). Only two species of fungi (*Aspergillus glaucus* and *Fusarium* sp.) were isolated from rice infested with RFB,

Fig. 7. Temperature of milled rice infested with *Tribolium castaneum* and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.



Top
Middle
Bottom
TEMPERATURE (°C)
WEEKS
30
31
32
33
34
35
36
37
4
8
12
16
20
24

and the percentage of kernels invaded was low. Both fungi were first detected in samples of rice taken from the top level of barrels at 8 wk. *Fusarium* was not found at any other time. The highest percentage of mold invasion recorded was 8% at 24 wk.

Fusarium spp., according to Christensen and Kaufmann (1974), are common field fungi on grain and will only grow in storage when the moisture content of the grain is in equilibrium with a relative humidity between 90 - 100%. The highest moisture content recorded in the T-system was 14.9% (top level after 8 wk) which is suitable for the *A. glaucus* group at 30°C (Agrawal et al. 1958; Christensen and Kaufmann 1974). Although the environment was suitable, little *A. glaucus* was found invading the rice during 24 wk in storage. Probably, RFB inhibited the development of *A. glaucus* and other storage fungi. Van Wijk *et al*. (1959) reported that confused flour beetle secreted a quinone which was relatively toxic to some storage fungi.

Loss measurements (Fig. 8; Tables A-5,6,7).

1. Flotation method. The average percentage weight loss found by floating off dust and kernel fragments increased gradually as storage period increased (Table A-5). Greatest loss (2.2%) was found at the end of experiment after 24 wk of storage. The greatest F-loss at each observation was found near the bottom compared to the other two levels. The amount of dust was greater near the bottom than at the other level, a factor that would influence measurement of loss using this method. Although, statistically, there was no significant correlation between F-loss and dust recovered (Table 1), a significant relationship was found with the time in storage (r = 0.84).

Table 1. Correlation among several factors in rice infested with *Tribolium castaneum* (Herbst) and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

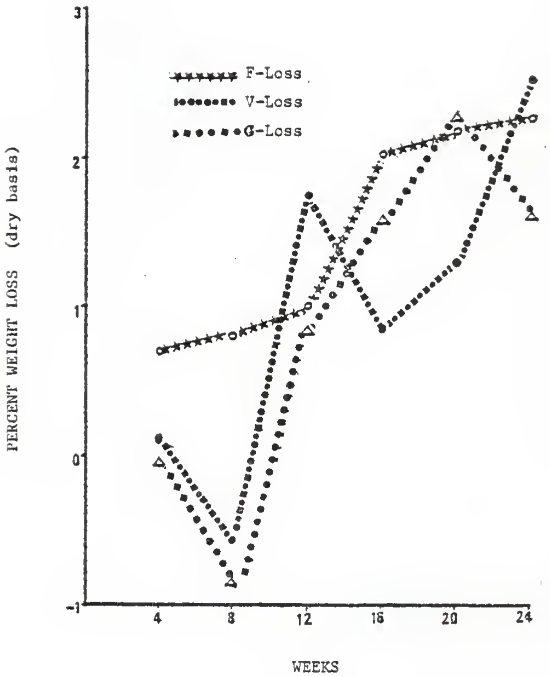
	F-loss	V-loss	G-loss	Dust recovered	Insect numbers	Moisture content	Temp.
Dust recovered	.652	.658*	.585*				
Insect numbers	.774	.607	.655*	.793**			
Moisture content	.535	.289	.359	.507	.821**		
Temperature	.471	.273	.139	.349	.718**	.816**	
Time in storage	.918**	.796**	.572	.829**	.869**	.647*	.560

F-loss with 6 degrees of freedom (d.f.), others with 12 d.f.

* : significant at P = 0.05.

** : significant at P = 0.01.

Fig. 8. Percent dry matter weight loss of milled rice infested *Tribolium castaneum* and stored at 29 $\pm$ 1°C and 70 $\pm$ 5% r.h. during 24 wk, measured by flotation , volumetric and gravimetric methods.


F-Loss
V-Loss
G-Loss
3
2
1
0
-1
4
8
12
16
20
24
PERCENT WEIGHT LOSS (dry basis)
WEEKS

Regression analysis using backward elimination, yielded the best equation for predicting the F-loss:

$$Y = 0.401 + 0.101\ X$$

Standard error of estimate 0.363

where Y = % F-loss

X = time in storage

The regression line representing the above equation as shown in Fig. 9.

2. <u>Volumetric method</u>. Moisture content and shape of kernels influenced the loss estimate using the volumetric technique. Although the effect of moisture had been minimized in calculations by using a dry matter basis, it's effect was still noticed. Samples at the 8 wk observation gained weight presumably due to increased m.c. Thereafter, weight losses were noted although the percentage did not increase greatly. The greatest loss (2.5%) was recorded at 24 wk (Fig. 8; Table A-6).

Correlation analysis indicated that there was a close correlation between V-loss and several variables including dust recovered, number of insects, and time in storage (Table 1). Correlation coefficients for all the factors were highly significant ($P = 0.01$). Regression equation for predicting the V-loss is as follows:

$$Y = -0.166 + 0.095\ X$$

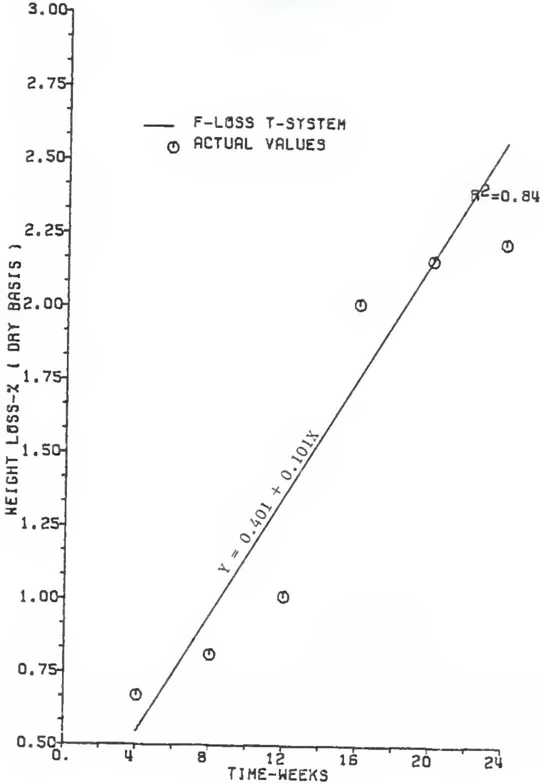
Standard error of estimate 0.607

where Y = % V-loss

X = time in storage

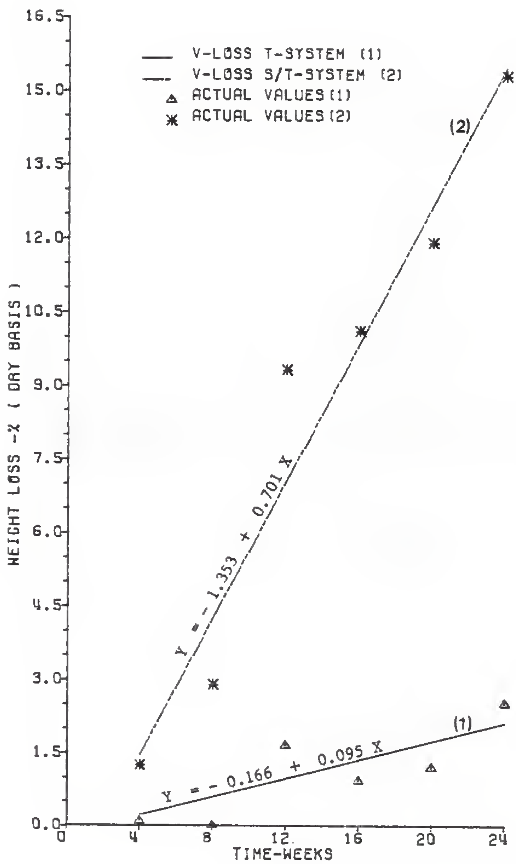
The regression line representing the above equation as shown in Fig. 10.

Fig. 9. Regression line of predicted dry matter weight loss of milled rice infested with <u>Tribolium castaneum</u> stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk, measured by flotation method.



3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
WEIGHT LØSS-% (DRY BASIS)
F-LØSS T-SYSTEM
ACTUAL VALUES
R²=0.84
Y = 0.401 + 0.101X
0.
4
8
12
16
20
24
TIME-WEEKS

Fig. 10. Regression lines of predicted weight loss of milled rice infested with (1) *Tribolium castaneum*, and of (2) *Sitophilus zeamais*/*T. castaneum* combined and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk, measured by volumetric method.



V-LOSS T-SYSTEM (1)
V-LOSS S/T-SYSTEM (2)
ACTUAL VALUES (1)
ACTUAL VALUES (2)
(2)
Y = - 1.353 + 0.701 X
(1)
Y = - 0.166 + 0.095 X
WEIGHT LOSS -% (DRY BASIS)
TIME-WEEKS
16.5
15.0
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0.0
0
4
8
12
16
20
24

Adams and Harman (1977) using a simulation storage model concluded that a volumetric method was accurate for predicting loss if the kernels of the grain were uniform.

3. Gravimetric method. Average percentage weight loss (G-loss) based on the weight of 1000 kernels of rice indicated that loss was variable between sampling levels and storage periods (Table A-7). The greatest average loss, recorded after 20 wk storage, was only 2.19% (Fig. 8).

Correlation analysis yielded a significant correlation between G-loss and dust recovered (Table 1).

The best regression equation for predicting the G-loss with two paris of variables is as follows:

$$Y = 32.220 + 0.043\ X_1 - 1.061\ X_2$$

Standard error of estimate for $X_1 = 0.011$ and $X_2 = 0.437$.

where Y = % G-loss

X_1 = number of insects

X_2 = temperature

The slight reduction of weight as a result of RFB infestation was almost unmeasurable, especially when volumetric and gravimetric methods were used. The flotation method, on the other hand, gave a slightly higher percentage of loss. Further studies using more observations and types of grains are still needed for validating this method.

Several workers have shown that the RFB did not seriously damage grain; however, RFB became a serious pest if associated with primary feeding insects, such as the rice weevil, lesser grain borer or Angoumois grain moth (Prevett 1971; Cotton and Wilbur 1974). McGregor

(1964) and McGaughey (1974) agreed that the fecundity of this species could be magnified by a high percentage of dockage and low degree of milling in rice. They believed that high populations would seriously damage rice.

Effect of Sitophilus zeamais on stored rice

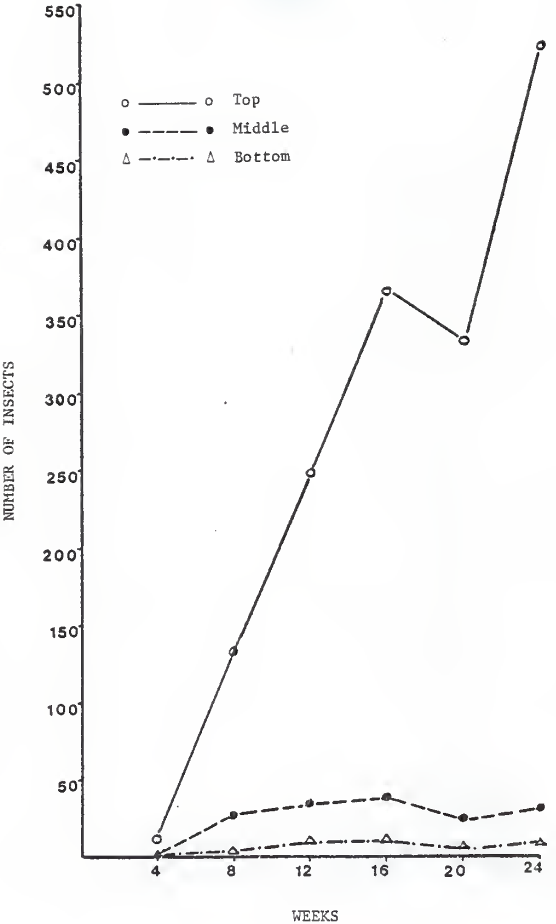
Number of insects (Fig. 11; Table A-1). The average population of MW in the barrels increased from 3.3/50-g sample at 4 wk to 188/50 g at 24 wk. At the 20 wk, the number of insects was slightly less than at 16 wk, but increased again by the last observation (Table A-1). With an average maximum density of 188 insects/50 g, or about 1 insect/19 kernels, an increase in numbers would still be possible according to Richards (1947). He noted that oviposition of Calandra spp. declined when population density reaching 1 insect/10 kernels.

Throughout the experiment the MW were concentrated in the top levels of the barrels. Few were recovered from bottom level. The greatest number in bottom level was recorded at 16 wk, with an average of 13/50-g sample compared to 377 and 40 for top and middle levels, respectively (Table A-1).

Using granary weevils in a small container of wheat, Surtees (1963a and 1964a) found this species concentrated at the surface and in the peripheral areas. Contrarywise, Howe (1951) using wheat placed in a steel tower, found that downward movement of grain weevils (he named them Calandra oryzae, pigmy strain, C. oryzae and C. granaria) was a natural one and only crowding and heat could drive them upward.

Sharangapani and Pingale (1957) using four species of stored product insects, reported that in wheat stored in bags and arranged in stacks, lesser grain borer, red flour beetle, rice weevil and Laetheticus oryzae (Wat.). Concentrations were influenced by the place at which insects were introduced. Insects introduced on the surface of the bags (in the stack) moved to the lower level, whereas insects introduced at the bottom were found more at the top.

Fig. 11. Number of insects per 50-g sample of milled rice infested with *Sitophilus zeamais* and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.



550
500
450
400
350
300
250
200
150
100
50
NUMBER OF INSECTS
Top
Middle
Bottom
4
8
12
16
20
24
WEEKS

In this study, the compactness of the rice may have influenced movement of the insect. After 16 wk storage, dust which had settled toward the bottom of the barrels may have inhibited the movement of insects to the lower levels. Fewer MW were found at the middle level that near the top, and the fewest near the bottom of the barrel (Table A-1). The grain became more compact and caked after molds started growing in peripheral areas.

Radiography. X-ray was used to detect the immature stages of MW and insect damaged kernels. Numbers of insects found were not as numerous as numbers of damaged kernels. The greatest number of immature stages was at 12 wk with 39/1000 kernels. Thereafter the numbers declined sharply to 9, 11, and 9 at 16, 20 and 24 wks, respectively (Table A-8). The overall mean was 18/1000 kernels.

Distribution of internal infestation appeared to be proportional to the number of adults counted from each sample. There were more immatures in the top level than the bottom or middle levels, except at the last sampling when number of immatures was slightly higher in the middle level. However, no significant correlation existed between number of adults and the number of larvae or pupae ($r = -0.03$).

Number of damaged kernels increased steadily after 8 wk and the greatest number (384/1000 kernels) was recorded at the last sampling. The upper levels were the most severely damaged compared with middle or bottom levels. The number of damaged kernels correlated significantly with the number of insects counted visually ($r = 0.76$).

Dust production. The total quantity of dust recovered increased for the first four sampling intervals but decreased in the 20 wk and 24 wk samples (Fig. 12; Table A-2). The most dust recovered was a mean of approximately 4 g after 16 wk storage. The amount of dust sieved from the top level increased along with the numbers of weevils up to 16 wk, and thereafter decreased although MW numbers increased. There was a tendency for dust to settle to the lower levels due to the activity of the increasing numbers of insects in the upper layer. After 20 wk storage, caked material (consisting of damaged kernels, dead insects, frass, mold and grain) was recovered from some samples. Moisture tests on dust indicated that the moisture content was 26 - 28%. At that moisture content, dust tended to clump with other materials. The decrease in amount of dust recovered after 16 wk was due to increased amount of dust incorporated into caked material.

It was expected that the total amount of dust would correlate directly with numbers of insects; however, there was no significant correlation ($r = 0.26$). This may be because of the inability to recover all the dust at last two sampling intervals.

Grain moisture (Fig. 13; Table A-3). Changes in moisture content (m.c.) of the grain infested with MW compared to the control were apparent after 12 weeks storage. Prior to that time, moisture changes reflected the establishment of equilibrium m.c. with the environment. Moisture content increased most rapidly in the bottom layer of rice in the barrel. After 24 wk, m.c. of the grain was 18.36, 18.02 and 16.84%, at the bottom, middle and top level, respectively.

Fig. 12. Amount of dust recovered from 100-g sample of milled rice infested with *Sitophilus zeamais* and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

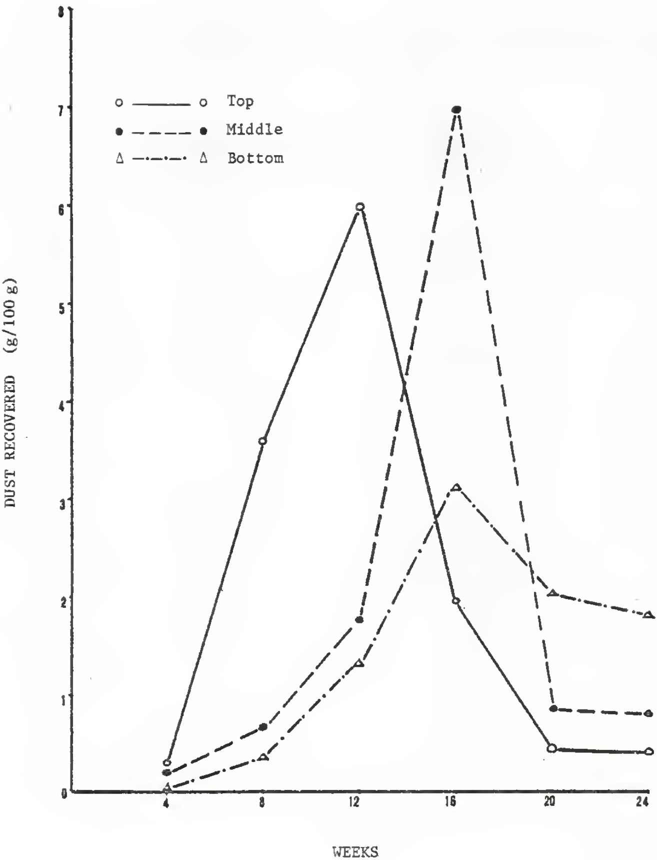
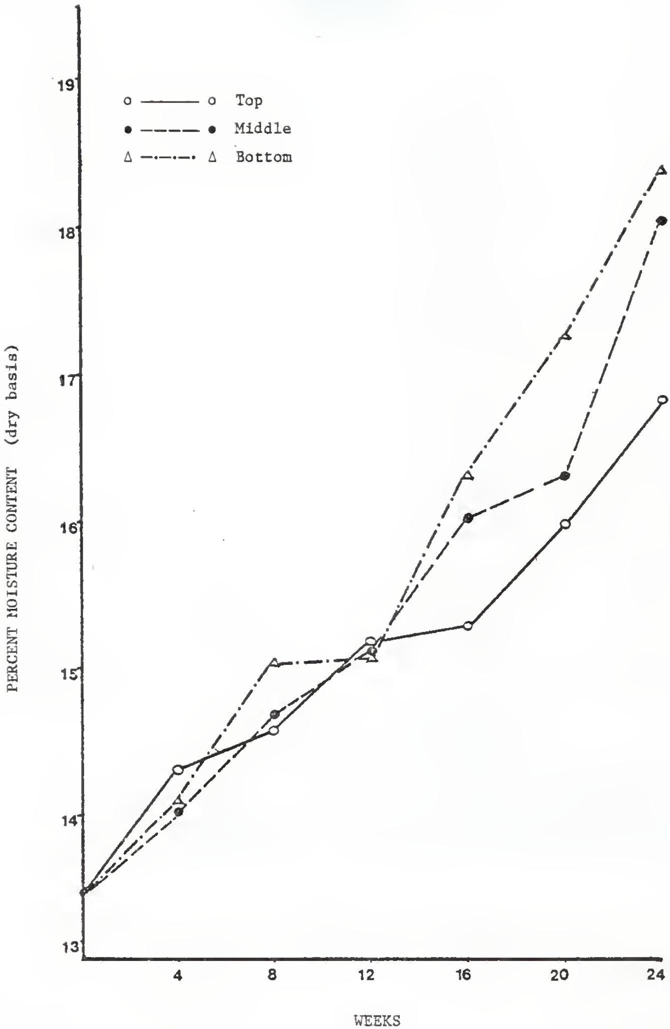

Top
Middle
Bottom
DUST RECOVERED (g/100 g)
WEEKS
0
1
2
3
4
5
6
7
8
4
8
12
16
20
24

Fig. 13. Percent moisture content of milled rice infested with *Sitophilus zeamais* and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.



19
18
17
16
15
14
13
PERCENT MOISTURE CONTENT (dry basis)
o —— o Top
• - - - • Middle
Δ -·-·- Δ Bottom
4
8
12
16
20
24
WEEKS

Increase in m.c. of stored grain as a result of insect infestation has been reported by several workers (Agrawal et al. 1957 and 1958; Hardman 1977) found that in wheat infested with rice weevils, the m.c. increased to 19.7% (initial moisture content 13.5%) after 60 days. There was agreement with Hardman's findings concerning the close relationship between moisture and temperature. Moisture content in this experiment increased as temperature increased; the two factors were highly correlated ($r = 0.96$).

Temperature (Fig. 14, Table A-4). The average temperature of rice in S-system was approximately 2°C above that of ambient air after 4 wk. The mean temperature increased steadily, reaching 36°C at the end of the experiment, whereas temperature in the control was almost unchanged.

Generally, the highest temperature at each sampling was at the top level where the insect population was highest. However, at the end of the experiment temperature in the bottom level was slightly higher than top level; perhaps mold growth was responsible. Screen-covered holes in the barrels might have provided enough air exchange to prevent the occurrence of hot-spots.

There was a significant correlation between temperature and other factors, including insect numbers, m.c. of the grain, time in storage, and number of damaged kernels (Table 2).

Mold invasion (Table A-10). Various species of fungi invaded rice in the S-system during the storage period. Invasion by molds began at 4 wk, but only three species were identified (*A. glaucus*, *A. candidus*

Fig. 14. Temperature of milled rice infested with Sitophilus zeamais and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

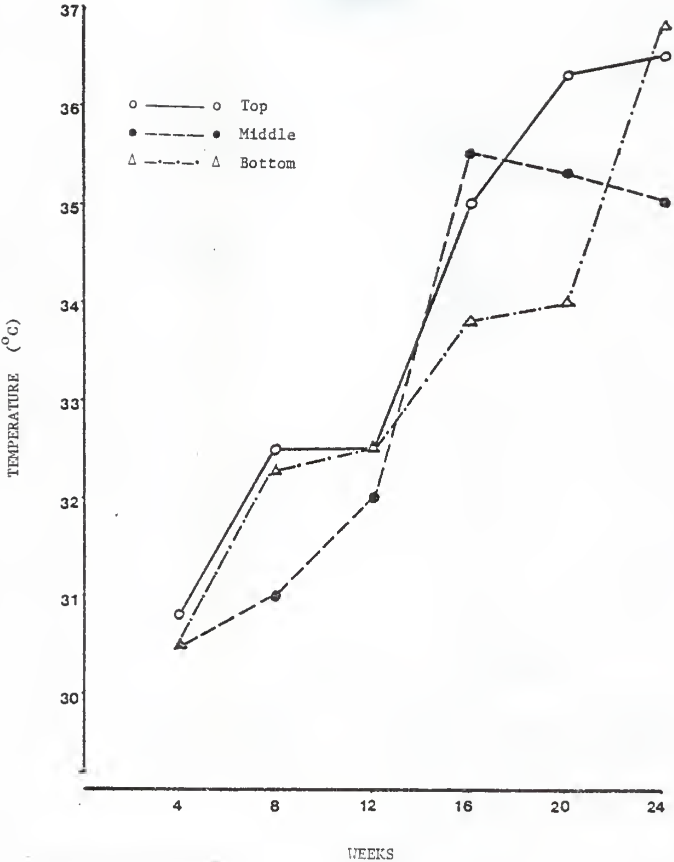

37
36
35
34
33
32
31
30
TEMPERATURE (°C)
Top
Middle
Bottom
4
8
12
16
20
24
WEEKS

Table 2. Correlation among several factors in rice infested with *Sitophilus zeamais* (Mots.) stored at 29° ± 1°C and 70 ± 5% r.h. during 24 wk.

	F-loss	V-loss	G-loss	Dust recovered	Insect numbers	Moisture Content	Temp.	Time in storage	Nos. of larvae/ pupae
Dust recovered	.905*	.308	.435						
Insect numbers	.419	.878**	.925**	.625*					
Moisture content	.118	.903**	.832**	.038	.720**				
Temperature	.352	.942**	.927**	.203	.812**	.935**			
Time in storage	.216	.959**	.908**	.158	.832**	.966**	.966**		
Number of larvae/pupae	-.071	-.309	-.246	-.256	-.034	-.299	-.297	-.238	
Numbers of damaged kernels	.175	.879**	.831**	.048	.756**	.957**	.942**	+.979	-.220

F-loss with 6 d.f. (degrees of freedom), others with 12 d.f.

* : significant at P = 0.05

** : significant at P = 0.01

and *Fusarium* sp.) with a low percentage of invasion. As grain moisture increased, the percentage of mold-damaged kernels increased. Between wk 4 and 12 *A. glaucus* predominated with level of kernel invasion ranging between 2 - 8%. In subsequent samplings, the percentage of *A. glaucus* declined and by 16 wk *A. candidus* outnumbered other species. The percentage of invasion was 36% at 24 wk. Other species were identified after 16 wk including *A. flavus*, *A. niger*, and *A. versicolor*.

Findings were in agreement with Christensen and Kaufmann (1974). *A. glaucus* predominated when moisture content was below 15% (Table A-3), and *A. candidus* appeared when moisture content reached about 16%. *A. flavus* was found after 16 wk especially in the bottom level of the barrels where dust accumulated. Moisture content of the dust at that point was high (26%) and was favorable for mold development.

Damaged kernels were more frequently invaded than sound kernels. Possibly, the damaged kernels had higher m.c. than sound kernels, or spores in the emergence holes were not destroyed during the disinfection process. Agrawal *et al.* (1957 and 1958) demonstrated the ability of granary weevils to spread spores of storage fungi in grain.

Culturing dust from each level at 16, 20 and 24 wk yielded mainly *A. glaucus*, *A. candidus*, and *Penicillium* spp. although other fungi including *A. versicolor*, *Fusarium* and yeasts were present. The same organisms were present. The same organisms were found in caked material recovered when barrels were emptied after the 6-month storage period.

Loss measurements (Fig. 15; Tables A-5,6,7).

1. Flotation method. The mean weight loss determined by the flotation method increased at each observation until 16 wk when the greatest loss was found. Thereafter weight loss was greatly reduced during the remaining 8 wk of experiment (Fig. 15; Table A-5).

The loss estimate determined by this method was influenced by the amount of dust recovered and number of damaged kernels because dust and damaged kernels were floated off during the washing process. The most dust recovered and the highest percentage of loss were recorded at 16 wk. The top and middle levels had greater losses compared to the bottom level until the 5th sampling (20 wk). Thereafter loss in the bottom level exceeded that in the middle level, but not that in the top level. The overall mean of weight loss recorded after 24 wk was 5.4% (range 3.36 - 9.72%). Cheigh *et al*. (1977), as cited by NAS (1978), found reduction in weight of polished rice was over 2% after washing. There was a high correlation ($r = 0.91$) between weight loss measured by the flotation method and amount of dust recovered (Table 2).

Using backward elimination analysis of nine factors, an estimate of weight loss in milled rice determined by the flotation technique could be predicted by the following equation:

$$Y = 3.57 - 1.597 X_1 + 0.059 X_2$$

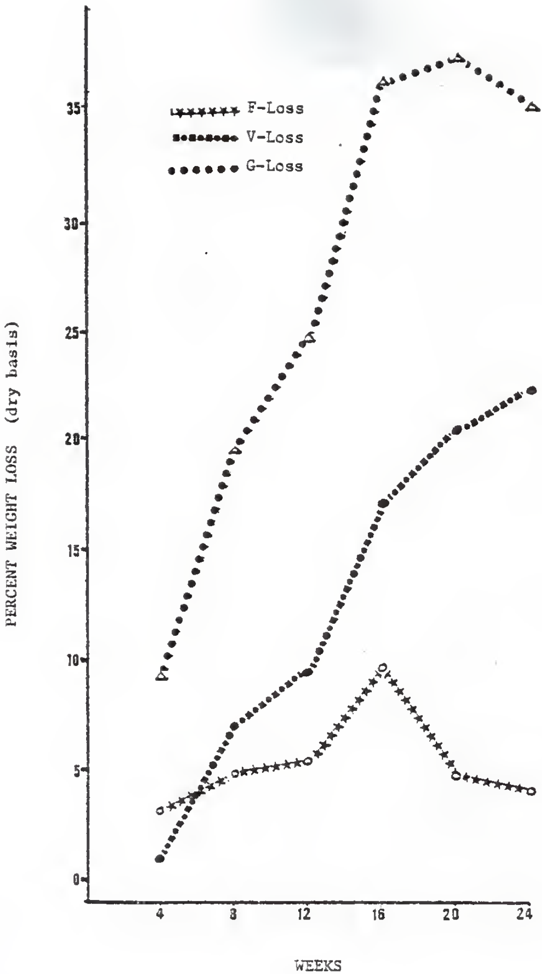
Standard error of estimate: 0.207 and 0.020 for X_1 and X_2, respectively.

where Y = % F-loss

X_1 = weight of dust recovered

X_2 = number of immature stages

Fig. 15. Percent dry matter weight loss of milled rice infested with <u>Sitophilus zeamais</u> and stored at 29 $\pm$ 1°C and 70 $\pm$ 5% r.h. during 24 wk, measured by flotation , volumetric and gravimetric methods.



F-Loss
V-Loss
G-Loss
PERCENT WEIGHT LOSS (dry basis)
0
5
10
15
20
25
30
35
4
8
12
16
20
24
WEEKS

2. <u>Volumetric method</u>. Measurable losses in rice infested with MW during a 24-wk storage period were found using the volumetric method. Appreciable weight loss occurred after 8 wk and increased at each sampling interval. The range of weight reduction per unit volume (50 ml) varied from 0.10 to 11.06 g at the middle level in the first observation and at the top level at the last sampling, respectively. The mean weight loss after 24 wk was 22.4%, whereas the overall mean loss during 24 wk was 12.9% (range 0.92 - 22.4%) (Fig. 15; Table A-6).

Estimates of weight loss based on the reduction of weight per unit volume were proportional to the number of damaged kernels. Correlation analysis depicted close interrelationship between the factors (r = 0.88).

Adams and Harman (1977) stated that the estimation of loss based on a volumetric technique was accurate as long as the grain was not heavily infested. They argued that severely damaged grain changed the overall volume of the grain and the estimate of weight loss would be misleading. However, they did not define the degree of infestation that would influence grain volume.

The overall mean of MW's per 50-gram sample was 101 or about 1 insect/41 kernels. Richards (1947) considered weevils in grain at a density below 1 insect/10 kernels were still able to oviposite and therefore increase the population. Based on Richards' assumption, rice in this experiment was not heavily infested, and therefore, the volumetric method was considered suitable for estimating loss.

Backward elimination analysis selected time in storage and number of damaged kernels as the factors which have the best interaction effect with

weight loss determined by the volumetric method. The equation for predicting the loss is as follows:

$$Y = -6.536 + 2.187\ X_1 - 0.050\ X_2$$

Standard error of estimate 0.233 and 0.010 for X_1 and X_2, respectively.

where Y = % V-loss

X_1 = time in storage

X_2 = number of damaged kernels

3. Gravimetric method. An increase in loss of 1000-kernel weight of rice infested with MW compared to control was recorded from 4 wk through the 20 wk in storage (9.35-37%)(Fig. 15). At 24 wk the weight loss slightly declined (35.0%). The overall mean loss was 26.9% (Table A-7). The loss was correlated to the number of damaged kernels ($r = 0.83$).

Weight loss measured by the gravimetric technique highly correlated to the number of insects, moisture content of the grain, grain temperature, time in storage and number of damaged kernels. The correlation coefficients of all five factors were highly significant at $P = 0.01$ (Table 2).

An equation for predicting the weight loss of milled rice measured by the gravimetric method, showed a multilinear relationship of number of insects and grain temperature:

$$Y = -63.572 + 0.103\ X_1 + 2.406\ X_2$$

Standard error of estimates 0.027 and 0.615 for X_1 and X_2, respectively.

where Y = % G-loss

X_1 = number of insects

X_2 = grain temperature (°C)

Effect of Sitophilus zeamais and Tribolium castaneum on stored rice

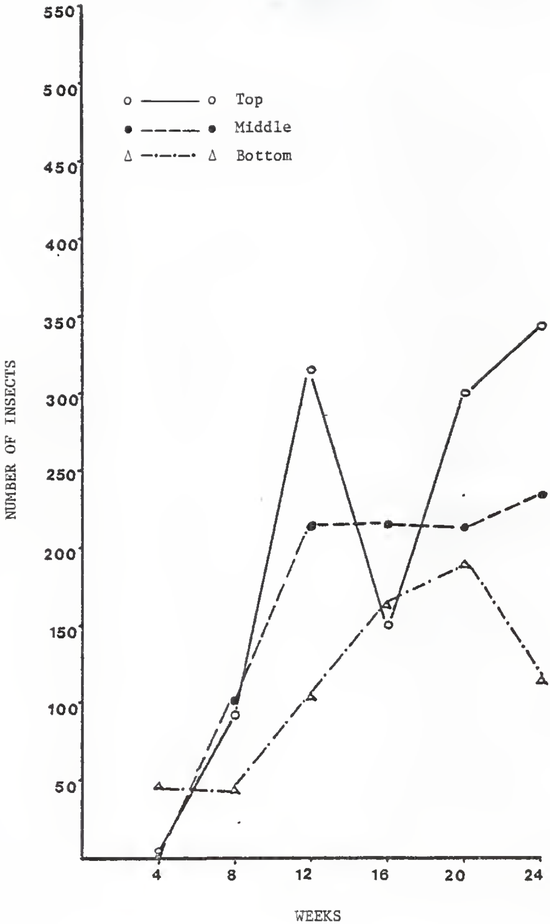
Number of insects. Insects in the barrels infested with MW and RFB (S/T-system) increased with time except for the 16-wk observation where the average population slightly decreased (Fig. 16; Table A-1).

At the first observation the number of RFB was higher than of MW and the difference became wider at the subsequent samplings (Fig. 17). The numbers of RFB increased throughout the experiment, whereas MW populations increased during the first three samplings to 70.3/50 g sample and then dropped sharply to 16.0 at 16 wk. The number of MW recovered changed little during the last half of the experiment. Apparently, *S. zeamais* was not able to compete with *T. castaneum*. RFB population appeared to increase faster than when MW were absent. The maximum number of RFB per 50-g sample was 217 or about 1 RFB/16 kernels compared to 1 RFB/43 kernels in the T-system. Presumably, RFB took advantage of dust and/or damaged kernels produced by MW.

There was also a possibility that RFB canibalized MW. Previous workers have reported that RFB prey upon egg and preadult stages of their own species or of another species when they live in the same media (Le Cato and Flaherty 1973; Le Cato 1975a, 1975b, 1975c). Le Cato (1975b) pointed out that both sawtoothed grain beetle and RFB produced more progeny and caused more damage to milled rice when they live together with rice weevils. However, there has been no report that RFB prey upon adult MW or on rice weevils when they occur in the same medium.

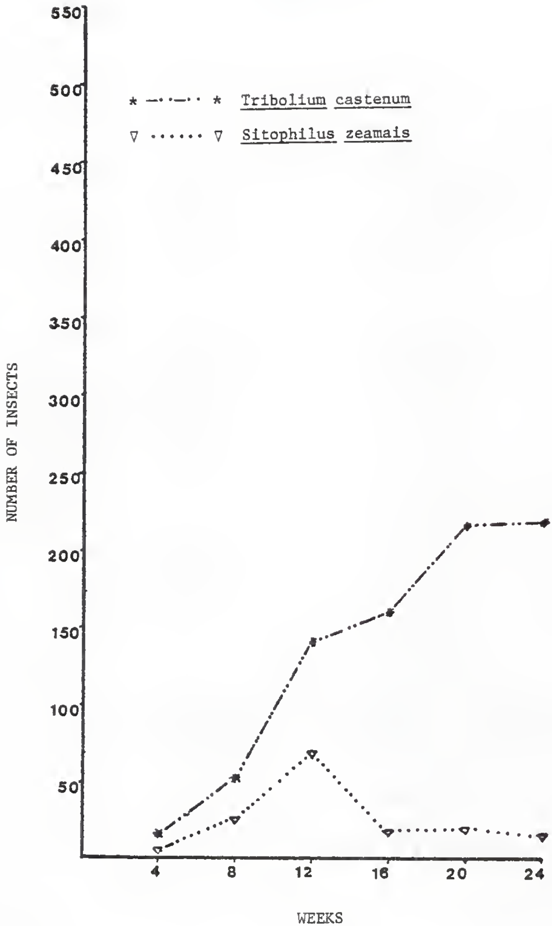
In contrast to these findings, Bronswijk and Sinha (1971) found that granary weevils outnumbered RFB in wheat media. They reasoned that

Fig. 16. Total number of insects per 50-g sample in top, middle and bottom levels of simulated bags of milled rice infested with both *Sitophilus zeamais* and *Tribolium castaneum* and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.



550
500
450
400
350
300
250
200
150
100
50
NUMBER OF INSECTS
Top
Middle
Bottom
4
8
12
16
20
24
WEEKS

Fig. 17. Average number of each species per 50-g sample of milled rice infested with both Sitophilus zeamais and Tribolium castaneum and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.



550
500
450
400
350
300
250
200
150
100
50
NUMBER OF INSECTS
* —·—· * Tribolium castenum
▽ ······ ▽ Sitophilus zeamais
4
8
12
16
20
24
WEEKS

temperature was the key factor in maintaining the high population of granary weevils. At the beginning of their experiment, temperature (between 25 - 28°C) was nearly optimum for granary weevil development. In this study, grain temperature in S/T-system ranged between 31-35°C which was nearly the optimum temperature for RFB (Birch 1953; Howe 1965a). Whether temperature or the presence of RFB was responsible for lowering the population of MW was not determined.

Distribution of each species in the barrels (simulated bags) was slightly different from that of S-system or T-system. The presence of RFB appeared to affect the dispersion of MW. As noted previously, MW alone tended to concentrate in the top levels. When RFB were present, the percentage of MW recovered at the top level was somewhat lower. It is possible that crowding by RFB occurred near the surface caused MW to move downward. Surtees (1965) noted the movement of rice weevils to the lower level when the surface level was crowded. Distribution of RFB was nearly uniform in the barrels.

Radiography (Table A-8). The average number of immature stages detected inside rice kernels varied from one sampling time to another. There was no trend observed during the experiment.

The greatest number of immature insects was recorded at 16 wk with an average of 42/1000 kernels, and the overall mean was 26.4/1000 kernels. Compared to the S-system the number of immature insects found in S/T-system was slightly higher since the number of larvae and/or pupae in S-system was only 18.4/1000 kernels.

As in rice infested with MW, the number of damaged kernels in the S/T-system steadily increased throughout the storage period and reached

214/1000 kernels. The overall mean of damaged kernels was 121/1000 kernels (range 9 - 214/1000 kernels).

With the exception of the 4 and 8 wk samplings, most immature insects were observed in the top level and fewer in the middle and bottom level, respectively. Number of damaged kernels was also higher in the top level compared with the middle or bottom levels, except in the 4, 8 and 12 wk samplings. During these first three samplings, middle levels had the greatest numbers of immature and damaged kernels.

Correlation between number of damaged kernels and other factors such as insect counts, m.c., temperature of the grain and time in storage was highly significant ($P = 0.01$) (Table 3).

Analysis of variance depicted no significant difference in number of insects or number of damaged kernels between S-system and S/T-system.

Dust production (Fig. 18; Table A-2). An increase of fine material recovered from each sample was noted at 8, 12 and 16 wk samplings. The greatest amount of dust was recovered at 16 wk (4.4 g/100 g sample). Adams (1970) infested single maize kernels with MW and after 37 days found the average overall weight of frass ejected by the larvae was 11.6 mg. He did not find the weight of frass ejected from each kernel to be significantly correlated with the number of insects. In this study, however, the number of insects per 50-g sample was significantly correlated with the weight of dust sieved from each sample (Table 3).

Compared to the S-system, the overall mean of dust recovered was slightly higher in the S/T-system, but the difference was not significant in analysis of variance. Bronswijk and Sinha (1971) found more dust in wheat infested with granary weevils and RFB than in wheat infested with

Table 3. Correlation among several factors in rice infested with Sitophilus zeamais (Mots.) and Tribolium castaneum (Herbst) and stored at 29° ± 1°C and 70 ± 5% r.h. during 24 wk.

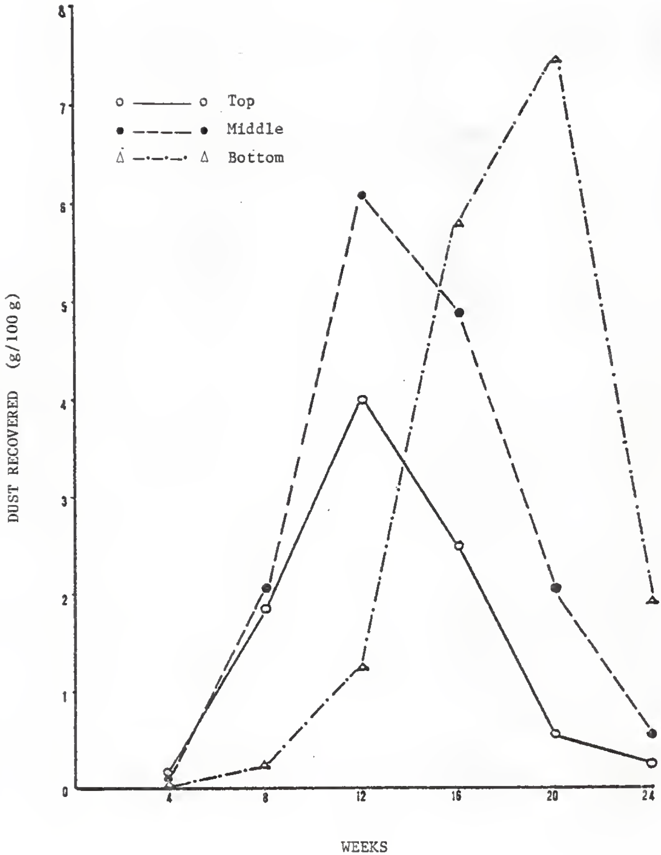
	F-loss	V-loss	G-loss	Dust recovered	Insect numbers	Moisture content	Temp.	Time in storage	Nos. of larvae/pupae
Dust recovered	.323	.340	.510						
Insect numbers	.931**	.917**	.935**	.582*					
Moisture content	.917**	.889**	.827**	.094	.792**				
Temperature	.799	.753**	.682**	.095	.700*	.907**			
Time in storage	.984**	.963**	.953**	.300	.890**	.953**	.853**		
Numbers of larvae/pupae	.317	.314	.379	.629*	.455	.213	.487	.314	
Numbers of damaged kernels	.951*	.939**	.934**	.227	.836**	.930**	.765**	.979**	.194

F-loss with 6 d.f. (degrees of freedom) others with 12 d.f.

* : significant at P = 0.05

** : significant at) = 0.01

Fig. 18. Amount of dust recovered from 100-g sample of milled rice infested with _Sitophilus zeamais_ and _Tribolium castaneum_ combined and stored at 29 $\pm$ 1°C and 70 $\pm$ 5% r.h. during 24 wk.


Top
Middle
Bottom
DUST RECOVERED (g/100 g)
WEEKS
0
1
2
3
4
5
6
7
8
4
8
12
16
20
24

Cryptolestes ferrugineus (Steph.) in combination with sawtoothed grain beetles, and most of the dust was concentrated at the bottom level of the barrels.

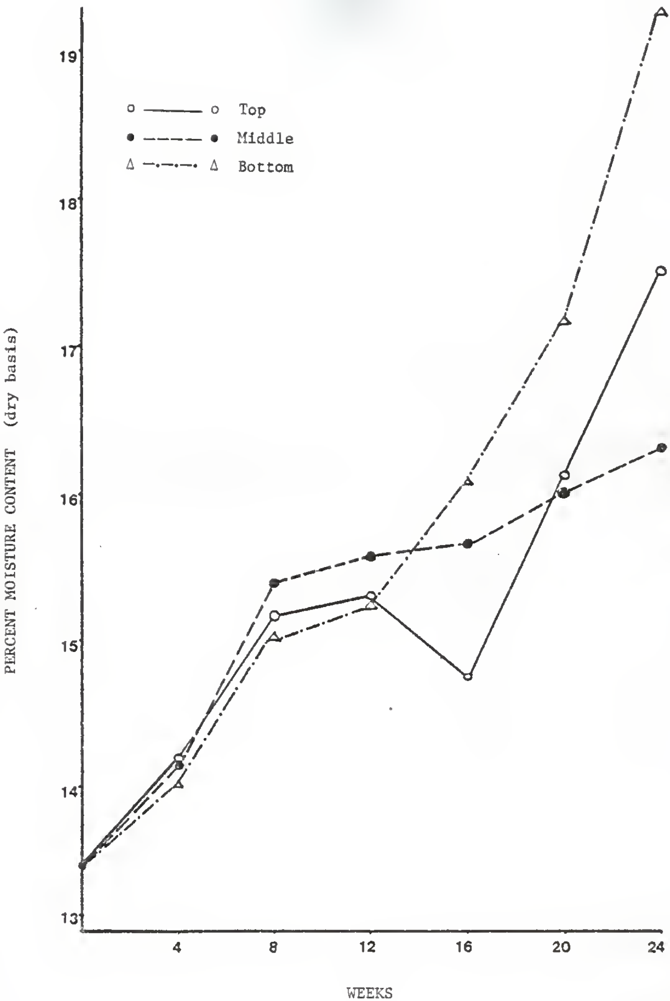
After 20 wk in storage the amount of dust recovered from the bottom layer decreased considerably, and less than 2 g/100-g sample was sieved at 24 wk, compared to more than 7 g/100 g at 20 wk. Caked material was found in the 20 wk and 24 wk samplings, especially near the sampling-port lids where air exchange was less than at other sites in the storage container. Formation of caked material reduced the amount of dust recovered. As in the S-system, dust recovery did not reflect the extent of insect damage at the end of experiment.

Grain moisture (Fig. 19, Table A-3). Appreciable increases in m.c. of rice infested with MW and RFB were recorded by the second observation at 8 wk. The highest m.c. reached was 19.3% in the bottom level at 24 wk.

Moisture content of the rice was closely correlated to temperature of the rice ($r = 0.91$); and m.c. increased linearly with the rise in temperature. Analysis of variance showed no significant difference in m.c. increases between rice in the S-system and S/T-system. However, moisture changes in S-system and S/T-system were significantly different compared to the T-system or in the control.

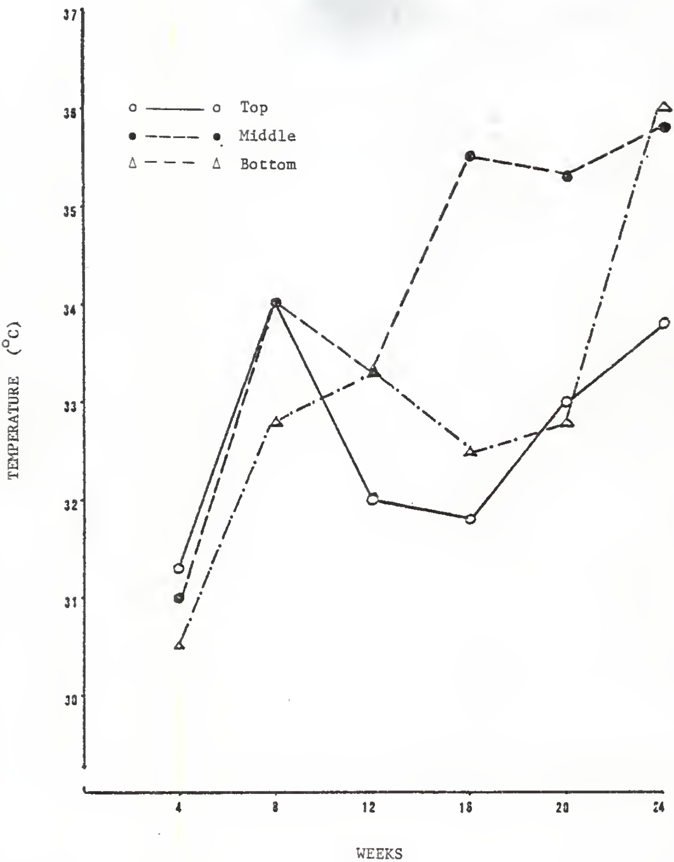
Temperature (Fig. 20, Table A-4). Average temperature of milled rice in the barrels infested with MW and RFB increased 1°C over that of the uninfested rice after 4 wk in storage and continued to rise, reaching 35.2°C at 24 wk. Temperature in the control barrels remained about the same as that of ambient air (30°C).

Fig. 19. Percent moisture content of milled rice infested with _Tribolium castaneum_ and _Sitophilus zeamais_ combined and stored at 29 $\pm$ 1°C and 70 $\pm$ 5% r.h. during 24 wk.



19
18
17
16
15
14
13
PERCENT MOISTURE CONTENT (dry basis)
o —— o Top
• – – – • Middle
Δ —·—·— Δ Bottom
4
8
12
16
20
24
WEEKS

Fig. 20. Temperature of milled rice infested with _Sitophilus zeamais_ and _Tribolium castaneum_ combined and stored at 29 $\pm$ 1°C and 70 $\pm$ 5% r.h. during 24 wk.



37
36
35
34
33
32
31
30
TEMPERATURE (°C)
Top
Middle
Bottom
4
8
12
16
20
24
WEEKS

The temperature difference from one level to another was 1 - 3°C at each sampling. Analysis of variance indicated no significant differences in temperatures at the three levels in the S/T-system and those in the S-system. Significant difference ($P = 0.05$) was noted between average temperature of S/T-system and S-system compared with the T-system or the uninfested rice. Temperature increases were linearly correlated to number of insects, moisture content of grain, time in storage and number of damaged kernels (Table 3).

Mold invasion (Table A-10). Low levels of mold invasion were detected in rice infested with MW and RFB after 4 wk storage. *A. glaucus* was found in both sound and insect-damaged kernels. A few *Fusarium*-infected kernels were found at the 4 wk sampling time. As moisture of the grain increased several species of fungi invaded the rice. The greatest infestation by *A. glaucus* occurred after 16 wk (12%); *A. candidus* and *Penicillium* spp. predominated at 20 and 24 wk. The percentage of *A. candidus* was 16 and 32% at 20 and 24 wk, respectively; whereas, *Penicillium* spp. infection was somewhat higher than that of *A. candidus* (16 and 36% at 20 and 24 wk, respectively).

Culturing caked material and dust from rice infested with MW and RFB yielded similar fungal populations to those found in rice infested with MW alone. Generally, there was no difference either in species or degree of fungal infection between the S/T-system and the S-system. Contrarywise, invasion in uninfested rice first occurred at 24 wk when *A. glaucus* had colonized 4% of kernels. The moisture content of the control (14.7%) provided suitable conditions for *A. glaucus* to grow (Christensen and Kaufmann 1974).

Loss measurement (Fig. 21; Tables A-5,6,7).

1. Flotation method. The percentage of weight loss measured by flotation gradually increased as storage time increased until 16 wk; thereafter, measurable loss decreased more than 50% (Table A-5). The average maximum reduction in weight was 10%, recorded after 16 wk in storage. The greatest percentages of weight loss were found in the middle and top levels.(Fig. 21).

Analysis of variance indicated no significant differences in total weight loss between rice infested with the combination of MW and RFB and MW alone; differences between S/T-system and T-system or the control were significant at P = 0.05.

The relationship between dust recovered and weight loss was not significant at P = 0.05, although significant correlation between weight loss and number of immature insects was noted. There was no close correlation between weight loss and number of damaged kernels or dust recovered which seemed inconsistent considering that washing and rinsing removed most of the damaged kernels and dust.

A regression equation for predicting percent weight loss was established:

$$Y = 1.707\ e^{0.038\ X}$$

Standard error of estimate 1.267

where Y = % F-loss

X = number of immature stages

The curve is shown in Fig. 22.

2. Volumetric method. The overall mean weight reduction after 24 wk storage of rice infested with a combination of MW and RFB was 8.46%

Fig. 21. Percent dry matter weight loss of milled rice infested with a combination of *Sitophilus zeamais* and *Tribolium castaneum* and stored at 29 $\pm$ 1°C and 70 $\pm$ 5% r.h. during 24 wk, measured by flotation , volumetric and gravimetric methods.

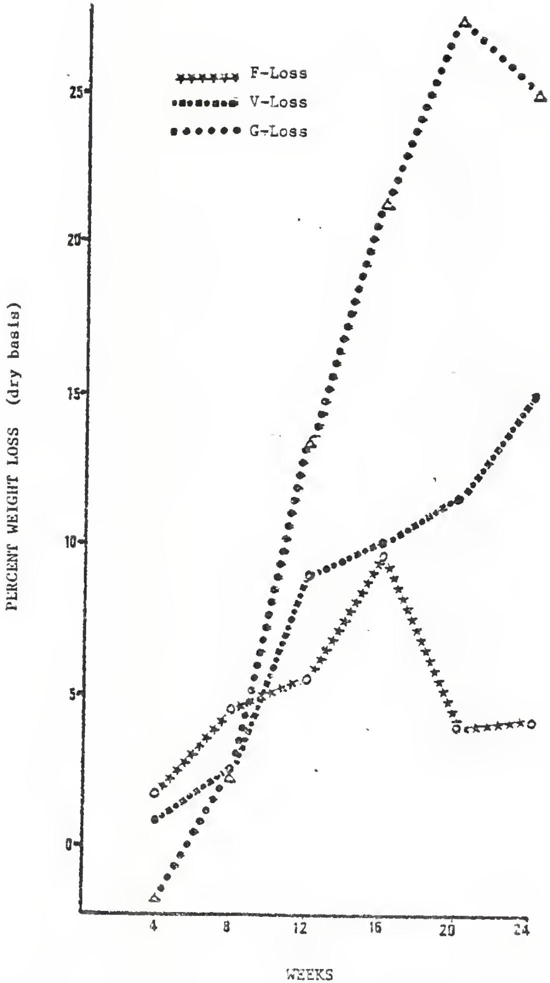
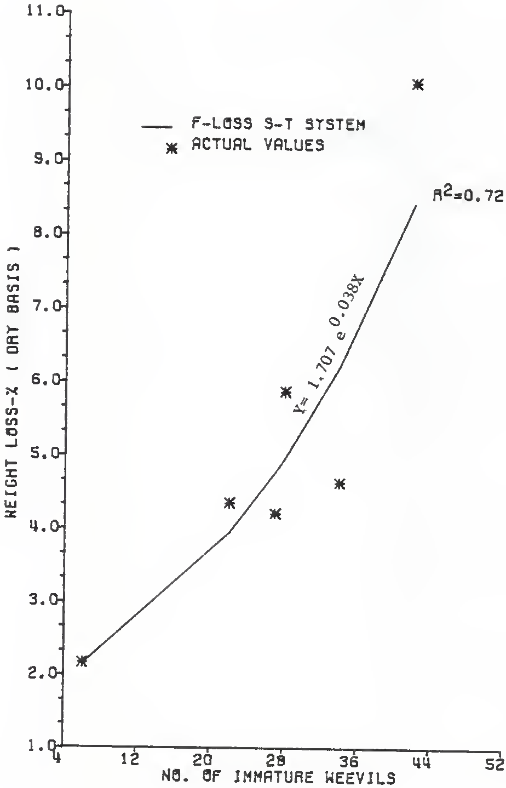

F-Loss
V-Loss
G-Loss
PERCENT WEIGHT LOSS (dry basis)
25
20
15
10
5
0
4
8
12
16
20
24
WEEKS

Fig. 22. Regression curve of predicted weight loss of milled rice infested with Sitophilus zeamais/Tribolium castaneum combined and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wks, measured by flotation method.



11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
WEIGHT LOSS-% (DRY BASIS)
F-LOSS S-T SYSTEM
ACTUAL VALUES
R2=0.72
Y= 1.707 e 0.038X
4
12
20
28
36
44
52
NO. OF IMMATURE WEEVILS

(range 1.24 - 15.30%) when determined by the volumetric method. This was about 4% lower than loss in the S-system measured by the same method (Table A-6).

In the S/T-system the overall mean population of MW for the 24 wk storage was 25/50 g rice and there were 121 damaged kernels/1000 kernels. The number of insects and number of damaged kernels in the S-system were almost twice that in the S/T-system with 101/50 g rice and 201/1000 kernels, respectively. The increasing population of MW brought about more damaged kernels, and consequently an increased percentage of weight loss measured by the volumetric method.

Loss in weight of a given volume corresponded linearly with several factors including number of insects, moisture content, temperature, time in storage and number of damaged kernels. The correlation coefficient for all the factors were highly significant at $P = 0.01$ (Table 3).

Analysis of variance indicated that the volumetric method gave a significant difference in weight loss between the S/T-system and S-system.

Multiple regression analysis was used to find the best equation for predicting the percentage of loss:

$$Y = -1.353 + 0.701\,X$$

Standard error of estimate 1.386

where Y = % V-loss

X = time in storage

The regression line of the above equation is shown in Fig. 10.

3. Gravimetric method. Thousand-kernel weight decreased during the storage period from 15.70 g at 4 wk to 13.57 g at 20 wk. The highest percentage of weight loss measured by the gravimetric method occurred at

20 wk. Thereafter the weight reduction decreased slightly from 27.5% to 25.2% at 20 and 24 wk, respectively. The overall weight loss was 14.86% and ranged from 2.85 to 25.24% at 8 and 24 wk, respectively (Fig. 21, Table A-7).

Percentage weight loss measured by the gravimetric method correlated with several factors including number of insects, m.c., temperature of the rice, time in storage and number of damaged kernels (Table 3). Multiple linear regression analysis used to predict the percentage weight loss yielded the following equation:

$$Y = 110.869 + 0.020\ X_1 - 3.805\ X_2 + 0.241\ X_3 + 0.154\ X_4$$

Standard error of estimates 0.010, 0.563, 0.163 and 0.039 for X_1, X_2, X_3 and X_4, respectively.

where Y = % G-loss

X_1 = number of insects

X_2 = rice temperature

X_3 = time in storage

X_4 = number of immature weevils

Analysis of variance revealed a significant difference in percentage weight loss between the S/T-system and S-system after 24 wk storage, based on the gravimetric method. Also, there was significant difference in weight loss between S/T-system and rice infested with RFB or uninfested.

Odor test

Results of multiple comparison difference analysis used in evaluating one aspect of rice quality deterioration are shown in Table A-11. Based on the odor of rice after 12 wk storage, panelists differentiated rice into three groups:

Group I - rice infested with MW and MW/RFB combined

Group II - rice infested with RFB alone

Group III - control rice

As the storage time lengthened, differentiation became more distinctive. In almost all observations rice in Group I was considered inferior to that of the reference (scores ranged from 6 - 9), indicating infestation by MW alone or in combination with RFB altered the odor of rice after only 12 wk storage. In Group II a moderate change was detected after 12 and 16 wk but rice became increasingly inferior to the reference sample as storage continued. Little change was noted in Group III until the last two samplings, when the uninfested rice became slightly inferior to the reference sample.

Because of subjectivity, the odor test alone would be difficult to apply in determining whether rice was still acceptable or not. Color and general appearance were important factors which panelists did not evaluate. The color and general appearance of rice in the S-system and in the S/T-system were very different from that in the T-system or in the control. After 16 wk color of most of the kernels in the S- and S/T-systems was light brown, especially in the top level, and became dark brown by the end of 24 wk. Rice in the T-system was white with a floury appearance,

while the control rice remained shiny, translucent white (normal appearance).

Preference of the panelists for rice had great variability. Some panelists preferred old rice with a specific odor and others preferred fresh rice. Scoring and overall results of the odor analysis were affected. Sensory analysis for evaluating the quality of food is rather subjective and influenced by several "extraneous errors" such as physical and mental conditions of the panelist, testing environment, nature of food preferences of the panelist and time when the test is conducted (ASTM 1968; Larmond 1970). However, a sensory test was useful for damage and loss evaluation, particularly early in the experiment, before the appearance of the rice was greatly affected by insect infestation.

Additional tests

Dust recovery (Table A-9a). Percentage of dust recovered from the T-system using the rotary mechanical sifter was only 0.85% and was lower compared to the S- or S/T-systems (1.33 and 1.79%, respectively). Generally the amount of dust recovered at the end of the experiment was higher than that manually separated in each observation. After 24 wk storage the amount of dust sieved from S- and S/T-systems were 1.0 and 0.89%, respectively. These comparisons confirmed the assumption that the amount of dust recovered in each probe sample did not reflect the actual dust production in each system. The formation of caked material in the S- and S/T-systems, and accumulation of dust at or near the bottom of the barrels reduced the amount of dust recovered.

Bulk density. Test weight, or bulk density test, was carried out at the beginning and end of the experiment using the standard method for density test of grain described by USDA (1957). The results revealed a considerable change of bulk density of rice in S- and S/T-systems. Bulk density at the beginning of the experiment was 64.3 lbs/bu in all systems. After 24 wk storage bulk density of rice in the T-system was 63.1 lbs/bu, whereas in S- and S/T-systems it was 59.7 and 60.2 lbs/bu, respectively. Rice in the control remained unchanged. The greatest comprehensive weight reduction per unit volume was recorded in the S-system (7.2%). Rice in this system was more severely damaged than in other systems and had a high percentage of damaged kernels. Hall (1972) noted that test weight of grain was affected by m.c. of the grain and the shape of the grain. Those two factors influenced space occupancy in the container and consequently the test weight of the grain.

Total weight loss (Table A-9b). Loss in dry matter weight of rice after 24 wk storage varied from an average of 2.25% in the control to 27.41% in the S-system. The combination of MW and RFB produced a lower average loss, 22.74%. Weight loss in both S- and S/T-systems were relatively large compared with the average in the T-system (3.70%). Loss was primarily attributed to formation of caked material, dust production, weevil consumption and metabolic activities of the insects and molds. RFB produced little dust and no caked material. MW alone or in combination with RFB produced a considerable amount of dust and promoted the formation of caked material. Although the amount of dust recovered in the S-system was less than that of the S/T-system, the percentage of weight loss was higher, primarily due to the amount of caked material.

Weight loss calculated by the difference between initial weight and end weight can give a good estimate of loss, although it may be rather difficult in a practical sense. In order to obtain accurate estimates of weight loss, this method should be supported with accurate records of moisture changes, dust recovered, and initial weight; otherwise results could be misleading.

Aflatoxin analysis. Rice from S- and S/T-systems and also dust and caked material suspected of containing aflatoxin (based upon results of mold tests, Table A-10) were analyzed. Although invasions of *A. flavus* were found during this experiment, only a trace (about 5 ppb) of aflatoxin B_1 was found in caked material from one replicate of the S-system. The highest percentage of *A. flavus* invasion was 16% in the S-system and it was found together with *A. candidus*, *A. versicolor*, *Penicillium* and yeasts (Table A-10).

A. flavus produces aflatoxin under a rather limited range of conditions compared to those required for growth of the mold. Aflatoxin produced by *A. flavus* is influenced by several factors including moisture, temperature, substrate and the presence of other species of fungi (Christensen and Kaufmann 1974; Christensen 1975). Even though conditions were favorable for aflatoxin production in rice during these tests, it is not known whether the small amount of aflatoxin B_1 produced was due to low capability of the specific *A. flavus* strain or because of inhibition. Christensen (1975) demonstrated in his laboratory that a strain of *A. flavus* capable of producing aflatoxin, when heavily inoculated into grain having favorable aflatoxin-producing conditions, failed to produce aflatoxin because other species of storage fungi present inhibited toxin production.

SUMMARY AND CONCLUSION

The extent of damage to stored milled rice by Sitophilus zeamais (Mots.). and Tribolium castaneum (Herbst), alone and in combination was measured in this study. Nine parameters of damage were examined at 4-wk intervals over a 24 wk storage period, and the relationship of each to weight loss measured by three methods was determined. Reasonably well-milled rice was stored at 29 $\pm$ 1°C and 70 $\pm$ 5% r.h. in duplicate 54-kg lots in metal barrels, modified to simulate bag storage.

RFB alone caused little damage compared to infestations of MW and of MW/RFB combined. RFB in the S/T-system was predominant and suppressed the population of MW. Although RFB developed well in the presence of MW, the insect had difficulty in maintaining its population when it was the only species in the rice medium.

Most insects were concentrated in the upper layer of the barrels, although there was a tendency for both RFB and MW to move downward when surface layers became crowded. Radiography confirmed that the top level was preferred by MW.

The number of damaged kernels detected by X-ray gave a good measure of the actual damage caused by weevil infestation. The number of damaged kernels related linearly to weevil populations.

Dust sifted from individual samples did not reflect the actual amount of dust produced by MW or RFB especially toward the end of experiment. Concentration of dust in the bottom level of the barrels and formation of caked material reduced the amount of dust recovered. However, dust production was a good parameter for weight loss prediction.

Moisture content of rice infested with RFB differed little from the uninfested rice throughout the storage period. In contrast, moisture contents of rice in the S- and S/T-systems increased appreciably. During the first 12 wk m.c. was highest in the upper level; thereafter, the bottom levels had the highest m.c.

Considerable increase in grain temperature was recorded in both the S- and S/T-systems, but temperature of rice in the T-system was not significantly different from that of the control. Highest temperatures were noted at the bottom levels in all barrels. Moisture content of rice in S- and S/T-systems was closely correlated with number of insects and grain temperature.

Mold invasion of kernels was influenced primarily by m.c. of the rice. Only _A. glaucus_ and _Fusarium_ sp. were identified in rice infested with RFB; the level of infestation of _A. glaucus_ was 8%. Rice in both the S- and S/T-systems was invaded by various fungi. During the first 12 wk in storage _A. glaucus_ predominated; later, _A. candidus_ and _Penicillium_ were predominant in the S/T-system while in the S-system, _A. candidus_ was the most abundant species. Other species found invading rice in the S- and S/T-systems were _A. flavus_, _A. niger_, _A. versicolor_, _Fusarium_ sp. and yeasts. The same species were found in dust and in caked material present in barrels infested with MW. At the end of the experiment, rice, dust, and caked material were analyzed for alfatoxin. Only one sample, caked material from the S-system, contained a trace amount (<5ppb) of alfatoxin B_1.

Dry-matter weight losses in simulated bag storage were obtained using three methods: flotation, volumetric and gravimetric. Weight losses in RFB-infested rice were low and were nearly the same regardless of the

method of measurement: 1.5% by flotation, 1.0% by volumetric (unit-volume weight), 0.9% by gravimetric (1000-kernel weight) methods. Regardless of method used, weight loss was greater in rice infested with MW alone than in rice infested by MW/RFB combined. Estimated loss was highest using 1000-kernel weight as the indicator and least when measured by the flotation technique. After 24 wk's storage the weight loss in rice infested with MW compared to the uninfested control was 4.3% measured by the flotation technique, 22.4% by standard volume and 35.0% by 1000-kernel weight. Compared in the same way, weight losses in rice infested with MW and RFB were 4.3%, 25.2% and 15.3%, respectively. Generally, when losses were measured by volumetric and gravimetric methods, losses increased as the storage period lengthened. However, using the flotation method, the greatest loss was found at 16 weeks with 10% weight reduction in both the S- and S/T-systems. The flotation technique was not as sensitive to weight changes caused by insect damage to the rice kernels, and when dust and insect frass became caked during the last third of the storage period, the material available for removal by flotation decreased.

Several factors were closely correlated with percent weight loss caused by insect infestation, as determined by flotation, volumetric and gravimetric methods.

In rice infested with RFB, flotation loss had a linear relationship with time in storage providing a practical method to estimate loss. The interrelationship of grain temperature and number of insects generated a multilinear regression equation to predict weight loss measured by the gravimetric method.

MW caused much damage and weight loss to rice. Only multilinear regression equations were able to give a reasonable loss assessment regardless of the method used to measure weight loss.

The combination of MW and RFB (S/T-system) produced damages and losses slightly lower than those found in the S-system. Time in storage had a linear relationship to weight loss using the volumetric technique. An exponential equation was fitted for predicting weight loss measured by flotation with the number of immature weevils as a parameter. Four parameters including grain temperature, number of insects, number of immature weevils and time in storage provided prediction of weight loss based on the 1000-kernel weight. The establishment of equations to predict weight losses was useful in a practical sense by showing that time-consuming work collecting insignificant data might be avoided. However, in order to test the validity of the various methods of measuring losses, further studies with a larger number of observations are needed.

Sensory analysis to detect odor changes as an indicator of quality deterioration showed that MW infestation alone or in combination with RFB gave an objectionable odor to stored rice by 12 wk, whereas RFB infestation did not alter the odor until rice was stored for nearly 20 wk. The odor test was useful for detecting quality degradation of rice during the storage period.

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APPENDIX

Table A-1. Insect numbers in milled rice infested with _Sitophilus zeamais_ and _Tribolium castaneum_ and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

Storage time (wk)	Level in Barrel	T-system		S-system		S/T-system					
		T. castaneum	Mean	_S. zeamais_	Mean	_T. castaneum_	Mean	_S. zeamais_	Mean	Total number	Mean
	Top(T)	9		10		6		1		7	
4	Middle(M)	7	7.3	0	3.3	0	16.3	0	2.0	0	18.3
	Bottom(B)	5		0		43		5		48	
	T	78		133		65		19		84	
8	M	40	46.3	27	55.3	52	53.0	51	24.7	103	77.7
	B	21		5		42		4		46	
	T	92		249		152		166		318	
12	M	48	55.3	35	98.3	176	142.0	40	70.3	216	212.3
	B	26		11		98		5		103	
	T	104		377		123		27		150	
16	M	55	66.3	40	143.3	201	160.3	14	16.0	215	176.3
	B	40		13		157		7		164	
	T	105		329		272		29		301	
20	M	63	65.3	25	120.0	187	215.3	27	19.0	214	236.0
	B	28		8		187		6		193	
	T	116		523		331		12		343	
24	M	75	71.3	32	188.3	206	217.3	33	15.7	239	233.0
	B	23		10		115		2		117	
Overall mean			51.9b		101.4a		134.0a		24.6c		158.9a

Overall means with similar letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Table A-2. Dust weight sifted (through of No. 40 sieve) g/100- g sample of rice infested with _Sitophilus zeamais_ and _Tribolium castaneum_ and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

Storage time (wk)	Level in barrel	T-system Dust weight (g)	T-system Mean	S-system Dust weight (g)	S-system Mean	S/T-system Dust weight (g)	S/T-system Mean	Control Dust weight (g)	Control Mean
4	Top(T)	0.045		0.284		0.116		0.018	
	Middle(M)	0.040	0.036	0.120	0.153	0.087	0.079	0.015	0.017
	Bottom(B)	0.023		0.055		0.033		0.018	
8	T	0.102		3.609		1.818		0.026	
	M	0.117	0.099	0.683	1.561	2.007	1.356	0.019	0.021
	B	0.077		0.391		0.244		0.018	
12	T	0.174		5.990		3.959		0.044	
	M	0.262	0.225	1.763	3.026	6.036	3.742	0.036	0.031
	B	0.238		1.324		1.230		0.014	
16	T	0.130		1.962		2.483		0.048	
	H	0.242	0.275	6.918	3.997	4.869	4.368	0.038	0.035
	B	0.452		3.110		5.752		0.020	
20	T	0.088		0.491		0.561		0.031	
	M	0.058	0.173	0.890	1.141	2.059	3.340	0.027	0.034
	B	0.374		2.043		7.401		0.043	
24	T	0.284		0.415		0.236		0.017	
	M	0.355	0.469	0.803	1.008	0.536	0.893	0.021	0.023
	B	0.767		1.805		1.907		0.030	
Overall mean			0.213b		1.814a		2.296a		0.027b

Overall means with similar letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Table A-3. Percent moisture content (wet basis) of the milled rice infested with <u>Sitophilus zeamais</u> and <u>Tribolium castaneum</u> and stored at 29 ± 1°C and 70% ± 5% r.h. during 24 wk.

Storage time (wk)	Level in barrel	T-system		S-system		S/T-system		Control	
		m.c.(%)	Mean	m.c.(%)	Mean	m.c.(%)	Mean	m.c.(%)	Mean
	Top(T)	14.05		14.32		14.20		14.47	
4	Middle(M)	13.89	13.89	14.04	14.16	14.17	14.14	14.45	14.34
	Bottom(B)	13.74		14.11		14.05		14.11	
	T	14.90		14.61		15.18		15.08	
8	M	14.76	14.83	14.72	14.79	15.40	15.22	14.83	14.88
	B	14.83		15.03		15.07		14.73	
	T	14.38		15.19		15.30		14.37	
12	M	14.54	14.50	15.13	15.14	15.59	15.36	14.36	14.39
	B	14.57		15.09		15.26		14.43	
	T	14.65		15.28		14.78		14.38	
16	M	14.57	14.61	16.00	15.87	15.68	15.51	14.42	14.42
	B	14.61		16.33		16.08		14.47	
	T	14.46		15.97		16.13		14.62	
20	M	14.82	14.71	16.32	16.52	15.99	16.43	14.63	14.64
	B	14.85		17.26		17.18		14.66	
	T	14.64		16.84		17.54		14.66	
24	M	14.88	14.81	18.02	17.74	16.30	17.70	14.66	14.67
	B	14.91		18.36		19.27		14.68	
Overall mean			14.56b		15.70a		15.73a		14.56b

Overall means with similar letter are not significantly different at $P = 0.05$ by Duncan's multiple range test.

Table A-4. Temperature (°C) of milled rice infested with *Sitophilus zeamais* and *Tribolium castaneum* and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

Storage time (wk)	Level in barrel	T-system Temp. (°C)	T-system Mean	S-system Temp. (°C)	S-system Mean	S/T-system Temp. (°C)	S/T-system Mean	Control Temp. (°C)	Control Mean
	Top(T)	30.3		30.8		31.3		30.3	
4	Middle(M)	30.3	30.2	30.5	30.6	31.0	30.9	30.0	29.9
	Bottom(B)	30.0		30.5		30.5		29.5	
	T	31.5		32.5		34.0		30.8	
8	M	31.5	31.5	31.0	31.9	34.0	33.6	30.0	30.2
	B	31.5		32.3		32.8		29.8	
	T	31.0		32.5		32.0		30.0	
12	M	30.8	30.9	32.0	32.3	33.3	32.9	30.8	30.4
	B	31.0		32.5		33.3		30.5	
	T	31.5		35.0		31.8		29.5	
16	M	31.8	31.2	35.5	34.8	35.5	33.3	30.3	29.8
	B	30.3		33.8		32.5		29.5	
	T	31.3		36.3		33.0		29.8	
20	M	31.5	31.2	35.3	35.2	35.3	33.7	30.0	29.9
	B	30.8		34.0		32.8		30.0	
	T	31.2		36.5		33.8		30.0	
24	M	31.5	31.3	35.0	36.1	35.8	35.2	29.8	29.9
	B	31.3		36.8		36.0		29.8	
Overall mean			31.3b		33.5a		33.3a		30.0b

Overall means with similar letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Table A-5. Percentage of weight loss determined by flotation method of rice infested with *Sitophilus zeamais* and *Tribolium castaneum* stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

Storage time (wk)	Level in barrel	T-system Weight loss (%)	T-system Mean	S-system Weight loss (%)	S-system Mean	S/T-system Weight loss (%)	S/T-system Mean	Control Weight loss (%)	Control Mean
	Top(T)	0.45		4.98		2.01		0.015	
4	Middle(M)	0.66	0.67	3.89	3.36	2.65	2.16	0.011	0.012
	Bottom(B)	0.89		1.20		1.83		0.010	
	T	0.65		6.56		3.83		0.045	
8	M	0.75	0.81	5.80	4.77	7.89	4.62	0.021	0.035
	B	1.02		1.95		2.12		0.040	
	T	0.74		7.71		4.19		0.070	
12	M	0.88	1.01	6.05	5.46	10.39	5.86	0.050	0.086
	B	1.40		2.62		3.00		0.130	
	T	1.77		10.81		8.13		0.091	
16	M	1.59	2.01	10.87	9.72	10.76	10.06	0.078	0.119
	B	2.68		7.49		11.28		0.187	
	T	1.61		6.91		4.12		0.080	
20	M	2.03	2.16	3.36	4.70	4.65	4.20	0.040	0.053
	B	2.84		3.84		5.00		0.040	
	T	1.60		6.00		4.01		0.060	
24	M	2.00	2.22	3.25	4.35	4.35	4.34	0.040	0.043
	B	3.05		3.79		4.66		0.030	
Overall means			1.48b		5.39a		5.21a		0.058b

Overall means with similar letter are not significantly different at $P = 0.05$ by Duncan's multiple range test.

Table A-6. Percentage of weight loss determined by volumetric method of milled rice infested with Sitophilus zeamais and Tribolium castaneum stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

Storage time (wk)	Level in barrel	T-system Weight loss (%)	T-system Mean	S-system Weight loss (%)	S-system Mean	S/T-system Weight loss (%)	S/T-system Mean
	Top(T)	0.80		1.91		2.36	
4	Middle(M)	-0.86	0.08	0.32	0.92	0.89	1.24
	Bottom(B)	0.32		0.54		0.48	
	T	-0.87		14.59		1.78	
8	M	-0.93	-0.33	4.29	6.97	6.96	2.89
	B	0.80		2.03		-0.06	
	T	1.78		18.14		10.52	
12	M	2.19	1.65	7.06	9.46	11.15	9.32
	B	0.97		3.17		3.30	
	T	1.03		29.44		10.62	
16	M	0.48	0.92	16.55	17.17	17.16	10.11
	B	1.26		5.51		2.54	
	T	2.29		34.43		11.07	
20	M	0.61	1.20	19.68	20.42	17.61	11.91
	B	0.71		7.45		7.05	
	T	2.95		35.91		17.40	
24	M	2.21	2.51	21.90	22.41	19.04	15.30
	B	2.37		9.43		9.47	
Overall mean			1.01c		12.89a		8.46b

Overall means with similar letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Table A-7. Percentage of weight loss determined by gravimetric method of milled rice infested with <u>Sitophilus zeamais</u> and <u>Tribolium castaneum</u> stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

Storage time (wk)	Level in barrel	T-system Weight loss (%)	T-system Mean	S-system Weight loss (%)	S-system Mean	S/T-system Weight loss (%)	S/T-system Mean
	Top(T)	-0.23		12.23		-1.21	
4	Middle(M)	-1.43	-0.08	10.19	9.35	-1.21	-1.63
	Bottom(B)	1.43		5.72		-2.48	
	T	-0.37		27.39		5.00	
8	M	-3.27	-0.92	15.69	19.47	3.05	2.85
	B	0.88		15.33		0.51	
	T	0.30		43.20		18.29	
12	M	0.37	0.78	13.74	24.07	19.47	13.73
	B	1.67		15.27		3.42	
	T	2.87		53.76		18.03	
16	M	0.14	1.57	36.11	36.19	32.74	21.42
	B	1.71		18.69		13.48	
	T	2.79		54.11		26.92	
20	M	1.62	2.19	39.19	37.29	41.22	27.54
	B	2.17		18.57		14.49	
	T	2.90		49.22		31.13	
24	M	1.20	1.57	34.26	35.05	41.38	25.24
	B	0.60		21.66		3.21	
Overall mean			0.85c		26.90a		14.86b

Overall means with similar letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Table A-8. Numbers of kernels with immature stages of _Sitophilus zeamais_ and numbers of damaged kernels per 1000-kernels as detected by X-ray.

Storage time (wk)	Level in barrel	S/system				S/T-system			
		Immature stages	Mean/ barrel	Damaged kernels	Mean/ barrel	Immature stages	Mean/ barrel	Damaged kernels	Mean/ barrel
	Top(T)	12		19		6		12	
4	Middle(M)	9	7.3	13	11	11	5.7	15	9.0
	Bottom(B)	1		1		0		0	
	T	84		132		41		58	
8	M	17	35.0	41	59.7	57	33.7	88	49.7
	B	4		6		3		3	
	T	88		361		55		175	
12	M	26	39.3	109	161.3	29	28.3	180	120.0
	B	4		14		1		5	
	T	11		467		97		291	
16	M	11	8.7	201	230.0	26	41.7	152	150.3
	B	4		22		2		11	
	T	21		728		69		296	
20	M	9	11.0	298	357.7	9	27.0	193	180.7
	B	3		47		3		53	
	T	6		418		48		299	
24	M	19	9.0	681	384.0	14	22.0	272	214.0
	B	2		53		4		71	
Overall mean			18.4A		200.6a		26.4A		120.6a

Overall means with similar letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Table A-9a. Weights (pounds) of dust and caked material recovered from each system at the end of the experiment.

System	Dust recovered (lbs)	Dust recovered Mean	Caked material (lbs)	Caked material Mean
T-system (1)	0.93		-	
		0.78		-
T-system (2)	0.63		-	
S-system (1)	1.16		18.84	
		1.22		16.66
S-system (2)	1.27		14.48	
S/T-system (1)	1.70		6.55	
		1.64		7.49
S/T-system (2)	1.57		8.43	
Control (1)	0.41		-	
		0.40		-
Control (2)	0.39		-	

Table A-9b. Weights (pounds - dry basis) of rice infested with Sitophilus zeamais and Tribolium castaneum stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

System	Initial weight (lbs)	Weight of samples[a] (lbs)	End weight[b] (lbs)	Weight loss (lbs)	Percent weight loss	Means of % weight loss
T-system 1	91.47	4.54	83.55	3.38	3.70	3.70
T-system 2	91.69	3.64	84.65	3.40	3.71	
S-system 1	91.47	4.60	61.08	25.79	28.12	27.41
S-system 2	90.83	4.34	62.25	24.24	26.69	
S/T-system 1	90.83	4.21	67.08	19.54	21.51	22.74
S-system 2	91.91	4.26	65.63	22.02	23.96	
Control 1	90.18	4.16	83.91	2.11	2.34	2.25
Control 2	90.18	3.66	84.57	1.95	2.16	

a Total weight of samples removed.

b Weight of rice after dust and caked material were removed.

Table A-10. Number of invaded kernels and percentage of mold invasion on rice infested with <u>Sitophilus zeamais</u> and <u>Tribolium castaneum</u> and stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk.

System	Storage time (wk)		A. glaucus No.	A. glaucus % invasion	Fusarium No.	Fusarium % invasion	A. candidus No.	A. candidus % invasion	Penicillium sp. No.	Penicillium sp. % invasion	A. flavus No.	A. flavus % invasion	A. versicolor No.	A. versicolor % invasion	A. niger No.	A. niger % invasion	Yeasts No.	Yeasts % invasion
T-system[a,b]	4	S[d]	0	0	0	0												
	8	S	1	2	1	2												
	12	S	2	4	0	0												
	16	S	2	4	1	2												
	20	S	3	6	0	0												
	24	S	4	8	0	0												
S-system[a,c]	4	S	0	2	0	2	0	2	0	0	0	0	0	0	0	0	0	0
		0[e]	1		1		1		0		0		0		0		0	
	8	S	1	6	1	4	0	2	0	0	0	0	0	0	0	0	0	0
		0	2		1		1		0		0		0		0		0	
	12	S	1	8	0	4	1	6	0	0	0	2	0	0	0	0	0	0
		0	3		2		2		0		1		0		0		0	
	16	S	0	2	0	0	4	24	0	2	0	2	0	2	0	0	0	2
		0	1		0		8		1		1		1		0		1	
	20	S	0	2	0	0	4	32	1	4	1	6	0	2	0	0	0	2
		0	1		0		12		1		2		1		0		1	
	24	S	0	2	0	0	8	36	2	12	2	16	1	4	0	2	0	2
		0	1		0		10		4		6		1		1		1	
S/T-system[a,c]	4	S	0	4	0	2	0	2	0	0	0	0	0	0	0	0	0	
		0	2		1		1		0		0		0		0			
	8	S	1	2	0	0	0	0	0	0	0	0	0	0	0	0		
		0	0		0		0		0		1		0		0			
	12	S	1	4	0	0	0	2	0	2	0	0	0	2	0	2	0	2
		0	1		0		1		1		0		1		1		1	
	16	S	2	12	0	0	0	4	1	8	1	8	0	2	1	2	0	2
		0	4		0		2		3		3		1		0		1	
	20	S	0	4	0	0	2	16	2	16	1	4	1	4	1	6	1	4
		0	2		0		6		6		1		1		2		1	
	24	S	0	4	0	0	4	32	8	36	1	8	1	10	0	2	1	4
		0	2		0		12		10		3		4		1		1	
Control[a,b]	24[f]	S	2	4														

a Rice from three levels in the barrels combined.

b Average of two replicates of 25 kernels each.

c Twenty-five sound and 25 damaged kernels.

d Sound kernels.

e Damaged kernels.

f No fungal invasion initially or until 24 wk.

Table A-11. Mean scores of odor test of milled rice infested with Sitophilus zeamais and Tribolium castaneum stored at 29 ± 1°C and 70 ± 5% r.h. during 24 wk as determined by nine panelists.

Storage time (wk)	Level in barrel	T-system	S-system	S/T-system	Control
	Top(T)	4.78 b	6.44 a	6.44 a	4.22 b
8	Middle(M)	4.89 b	6.00 a	6.00 a	4.00 c
	Bottom(B)	5.22 a	5.78 a	5.56 a	3.89 b
	T	6.67 b	7.67 ab	8.22 a	4.78 c
12	M	7.00 a	7.89 a	7.67 a	5.89 b
	B	6.22 b	8.11 a	7.89 a	5.33 b
	T	8.33 a	8.56 a	7.33 b	5.44 c
16	M	7.67 a	7.33 a	7.67 a	5.67 b
	B	7.11 b	8.11 a	8.22 a	5.78 c
	T	7.33 b	7.89 a	7.78 a	5.44 b
20	M	7.44 ab	8.33 a	7.44 ab	6.56 b
	B	7.56 a	8.56 a	8.33 a	5.33 b
	T	6.33 b	7.89 a	8.00 a	5.33 c
24	M	7.11 b	8.33 a	8.67 a	5.78 c
	B	7.22 bc	8.33 ab	8.56 a	6.11 c
Overall mean		6.75	7.69	7.65	5.32

Average of scores of nine panelists.

Scoring value: 1 = extremely better than R (reference sample)
2 = much better than R
3 = moderately better than R
4 = slightly better than R
5 = equal to R
6 = slightly inferior to R
7 = moderately inferior to R,
8 = much inferior to R
9 = extremely inferior to R

Means of each level from each observation with similar letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Overall means connected with line are not significantly different at P = 0.05 by Duncan's multiple range test.

Appendix B-1 Flotation method

<u>Flotation method</u>

Washing rice prior to cooking is the common practice in rice-eating countries in order to eliminate undesirable materials, such as dust, dead insects and some damaged kernels. This practice was adapted as a method for determining dry matter weight loss.

A 50-g sample of known m.c. was put into a 1000-ml glass beaker and 500 ml tap water was added. Rice was stirred thoroughly. Tap water was added so that suspended material (dust, insects and some damaged kernels) was floated off. Water was poured off, and the process was repeated twice. Then rice was drained, spread in a thin layer and allow to air-dry at room temperature for 24 hr. Samples were weighed and the m.c. determined using air-oven method described for whole grains (130°C for 19 hr). Weight loss was calculated on dry-weight basis.

Appendix B-2 Questionnaire used for the odor test

NAME: ____________________ DATE: ____________________

Questionnaire

You are receiving samples of rice to compare for the odor. You have been given a reference sample, marked R, to which you are to compare each sample. Test each sample: show whether it is better than, comparable to, or inferior to the reference. Then mark the amount of difference that exists.

Sample :	______	______	______	______
Better than R :	______	______	______	______
Equal to R :	______	______	______	______
Inferior to R :	______	______	______	______

AMOUNT OF DIFFERENCE:

None :	______	______	______	______
Slight :	______	______	______	______
Moderate :	______	______	______	______
Much :	______	______	______	______
Extreme :	______	______	______	______

COMMENTS:

Any comments you may have about the odor of the samples, please write down here.

EXTENT OF DAMAGE TO STORED MILLED RICE

BY INSECT INFESTATION

by

MULYO SIDIK

B.S., BANDUNG INSTITUTE OF TECHNOLOGY, 1972

BANDUNG, INDONESIA

AN ABSTRACT OF A MASTER'S THESIS

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Losses and damages to milled rice infested with maize weevils (MW), *Sitophilus seamais* Mots. and red flour beetles (RFB) *Tribolium castaneum* (Herbst), alone and in combination were investigated. Medium grain Nato variety rice, reasonably well-milled with 25% broken kernels, was infested and stored in barrels simulating bags. Nine parameters of damage were assesed at 4 wk intervals over a 24 wks period. Quantitative losses were measured by flotation, volumetric, and gravimetric methods.

The RFB population increased slowly during the storage period and caused little damage to the rice in terms of dust production, moisture content (m.c.) changes or mold invasion. No appreciable weight loss was detected regardless of method used.

MW alone and in combination with RFB caused significant losses and damage. Increased numbers of insects were closely correlated with increased rice m.c., temperature, and numbers of damaged kernels. MW's decreased markedly after 16 wk in the presence of RFB, whereas, RFB multiplied well as the beetles probably utilized dust produced by weevils for food. Highest m.c.'s and largest quantities of dust (composed chiefly of insect frass and kernel fragments) were found near the bottom level of the barrels.

Increase in m.c. was followed by invasion of kernels by a succession of fungi: *Aspergillus glaucus* was predominant after 12 wk storage but was replaced by *A. candidus* and *Penicillium* spp. by 16 wk. In rice infested with MW alone *A. candidus* was predominant. Caked material and dust found by 20 wk yielded *A. glaucus*, *A. candidus*, *A. versicolor*,

A. niger, yeasts and *Penicillium* spp. A trace amount of aflatoxin B_1 (5ppb) was detected in one sample of caked material from MW infestation.

Quantitative measurement of dry matter weight loss caused by MW and MW/RFB infestations ranged from 0.92 to 37.29% with an inconsistency in the degree of loss shown by the three methods used. Single and multiple linear regression equations were established to predict weight losses.

Quality deterioration was assessed by a panel of nine persons which compared the odor of rice samples from three locations in each combination of infestation and control with a reference sample stored at 4°C. Multiple comparison difference analyses indicated that MW alone and in combination with RFB altered the odor of rice by 12 wk, whereas RFB did not change the rice odor until after 20 wk storage. Odor of non-infested control rice did not differ significantly from the reference.